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Concentration of specific biogenic amines in ventricular CSF of type A and B Parkinson's disease patients on Sinemet

Justin Robert Ahlman

CONCENTRATIONS OF SPECIFIC BIOGENIC AMINES IN VENTRICULAR CSF OF TYPE A AND B PARKINSON'S DISEASE PATIENTS ON SINEMET

A Thesis

Presented to the

Faculty of

California State University,

San Bernardino

In Partial Fulfillment

of the Requirements for the Degree

Masters of Science

in Biology

By

Justin Robert Ahlman

June 2001

FIRST NATIONS TRIBAL LIBRARY

AND

SOCIAL RESEARCH CENTER

A Project

Presented to the

Faculty of

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In Partial Fulfillment

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Master of Arts

in

Interdisciplinary Studies

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by

Pamelalee Bailey-Shimizu

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Approved by:

[redacted]

David Decker, Chair, Sociology — 6/16/00 Date

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Elsa Valdez, Sociology

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Michael LeMay, Political Science

ABSTRACT

This writing covers one full year in time. The project was to establish a non-profit center for Native Americans in San Bernardino. I am describing the work and knowledge I acquired alone the way. What I started has changed my life. I have in that year found a direction and goal. All the years of education will go into developing a center to enhance the educational opportunities and community cohesiveness in San Bernardino. Like everything I have done in life the center will be about Native Americans and for all people regardless of race. Shakespeare in Hamlet said "to thine own self be true" with that in mine I am a Native American and what I do; I do on the "Red Road". Aho!

ACKNOWLEDGMENTS

My husband, Mori Shimizu has supported and reminded me to finish on time. I tended in the past to linger to long at school. My oldest daughter Shelley has been my best school partner. We spent several hours on the phone talking about test and papers. My Grandchildren are my inspirations for making new family traditions. I expect every Grandchild to use education to better the lives of their Grandchildren.

I have always been blessed with the quality of people who have nurtured my dreams of education. Even those who tried to stop me made me stronger and more determined. I don't believe there was a better Sociology Department for me.

Dr. David Decker, Dr. Elsa Valdez, Dr Michael LeMay, Dr. Patty Little, Dr. Cecila Julag'ay, George Gibbs, Dr.'s Tom and Lily Rivera, Gill Navarro, Janice White, Dean Sandra Kamusikiri own a piece of my heart and my degree.

For over fifty years I have tried to be an educated Indian, and thanks to Dr. James Fenelon, I know what a

classy, well educated Indian sounds, acts and writes like, for the betterment of Native American people and inclusive of all people. He and his family are wonderful role models.

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CHAPTER ONE

RHYME AND REASON

When I initiated the First Nations Tribal Library and Social Research Center, the plan was to offer to the San Bernardino Valley a comfortable center where Native Americans could network and where other people could learn about Native Americans. I already understood a little about being an "Urban Indian" as I had been born and raised in the city, but I was a Native American. That is, my roots were Native American, but having been raised in an urban environment, a great deal of the dominant culture had been forced onto my family.

Because of this forced acculturation, my own roots were denied me. However, from an early age I have identified strongly with my Native American roots. Though I am an "Urban Indian" my ancestors' blood speaks to me and as an adult I have learned to listen and - finally to be comfortable with whom I am.

A respected Elder once told me Urban Indians are "Feather People." What she meant was we have no roots so we

float on each wind looking for lost roots. In a sad way she was right. We know we are not whole. As much as we may desire it, we can never regain the true essence of cultural timing and orientation. Still in heart and blood we are Native American. We may be a century removed, but we are alive and well.

Currently there are more Native Americans living off the reservation than on it. However, as Native America grows in numbers outside of the reservation, Urban Indians are becoming more involved in the urban lifestyle. Living on the fringes of mainstream America, Urban Indians have increasingly become more invested in dominant core culture. Urban Indians have lost most of their Native cultural capital and replaced it with bits and pieces of "tradition" that have been pieced together from different tribal entities. In this way, it is not unusual to find an Apache like myself who can speak a few words of Lakota and Navajo, but none of the Apache dialect.

Although there is not currently a large population of visible Native Americans functioning in the upper middle class and above, there is a growing number moving from poverty into lower middle class positions. There has been a movement among Urban Indians to become functioning members within the dominant core system - a system that is primarily foreign and hostile. For these reasons, this system is uncomfortable for Native Americans living off the reservation.

In the past the answer to the "Indian Problem" was always to make them an exception, confined to controlled areas, isolated from mainstream America. Starting in the 1950's there were thousands of Native Americans relocated into large cities, with the assumption that they would become assimilated. This government program of forced assimilation was deemed a failure by the early 1970's. By this time thousands of Native Americans were "born and raised" in the off reservation style. Relocation made Southern California the second largest state in Native American population. In the year 1999 the official

government records showed slightly over half a million Din'e (Navajo) located in Southern California. Because reservation life no longer was comfortable to these Urban Indians most chose to remain in the city.

When Native Americans find themselves in the city there are numerous problems. On Reservations reality may be rough, but there is a strong sense of community, tradition, or cultural roots. Community and tradition are left behind when Native Americans are city bound. The only obvious tradition in the city seems to be the tradition of Native Americans being unemployed or working in lower paying jobs. In California the close resemblance between Native Americans and Latinos can increase the pressure to lose the Native identity. It has been well documented that the Mexican and other Latin American countries have Euro- centric forms of government, which enforce a hierarchical society with the indigenous people on the bottom rankings.

California now has "Indian Bingo and Gaming." Though gaming has meant some Indians are no longer in poverty, those Native American groups that are involved in gaming and

making money are suffering some unforeseen problems. A large number of problems have landed square on the shoulders of the poor urban Native Americans. Even though only a very small number of Indians actually receive benefits from gaming revenues, the non-Indian public often thinks most or all Indians are rich. In the not so distant past, Native Americans were perceived and treated as wards of the federal government and not capable of managing their own fiduciary responsibilities. Today, the monies that had been held in trust are missing and our United States President has stated that there is more money missing from the Bureau of Indian Affairs than there is in the national debt. Nobody is rushing to return this money. Senator Slate Gordon from the state of Washington has written several bills denying any future fiduciary obligations of the United States government to the Native Americans for past injustices. So far, he has not been successful, but he is widely recognized by Native America as one of the most dangerous racists in Congress.

It is funny how Native America went from "vanishing" to

being expected to respond to the financial problems of all Indians in the United States. None of that is true. Today Native Americans are the fastest growing ethnic group in the United States. Some of this growth is due to the fact that, since there is now money, there is legitimacy. The more affluent Native Americans are so far small in numbers and have so much to deal with they can't possibly answer the needs of millions of Urban Indians. It is like people expecting Donald Trump to make all Euro-Americans his problem because he's rich. The new money has in many ways made the divide between "Rez Indians" (those on reservation or close to the reservation) and the Urban Indians almost impassable.

Tribal elders who are trying to write grants (for example for non-profit organizations) to better their people are being challenged by mainstream politicians as to why they would need any money. It is common knowledge that Orange County, which is arguably the richest county in southern California writes for such grants on a regular basis. All non-profit organizations that serve people

survive by writing grants. Since that is the American way, why would it be wrong for Native Americans to now follow suit?

In order to serve my community the best, I needed a center where community activists could meet and educate people. For funding and community involvement we needed the local politicians to see our center as a source of pride, a place to be identified with. Sometimes that is a tricky mix, but if successful it means we can work within the legal system to make relevant change for the community. The center needs to be a place where we can teach voting rights and political involvement. As Native America learns to assert itself, having political representatives will be important. Already there is a new and growing Native American Democratic Caucus.

My dream was to have a place that Natives Americans could go to and gain an understanding of their civil rights. I also knew that many Native Americans do not understand how to effectively use most bureaucratic systems, like Social Security or the Department of Rehabilitation. My life had

taught me how to use these systems. I knew that I could pass this knowledge on. As a child I lived in foster homes as well as an orphanage. As an adult I have been a welfare mother, and now I am on Social Security and finishing my education with the help of the Department of Rehabilitation. I know bureaucratic paper work.

At school while getting my bachelors degree in sociology I studied why Native Americans did not finish their course studies and how the education system had not been a positive influence for Native Americans. Many other cultural groups have had problems with poverty, being away from "home," and with inadequate education. However, Native Americans have all of these problems and more. In addition to the above problems Native Americans are always in such small numbers in educational communities that comradery is almost impossible. The most debilitating problem of all is probably the fact that all levels of education in the United States continues to perpetuate a racist curriculum. Columbus could not have "discovered" America if people and their cultures were already thriving. Disregarding this fact, the

United States educational system clings dearly to this myth.

I have been the Executive Director of the San Bernardino branch of the American Indian Movement since 1997. This work has given me several reasons to want to study more about the laws that govern Native Americans. The American Indian Movement is a national civil rights organization. One of the little known facts about the American Indian Movement is that it is also a religious statement in that all members are devoted "Traditionalists." The term Traditionalist refers to Native Americans who follow the spirituality of the old ways. In the old tradition "AIM'sters (hard core American Indian Movement members) are a warrior society. They have become modernized in that their weapons are political actions like demonstrations and lawsuits. If registered with a federally recognized Nation, Native Americans have a very different legal standing in America. The laws of America are so twisted and change so often for Native Americans that the common person and sometimes even well educated people have no idea what the law really says. My idea was to offer a

large range of educational opportunities and also provide a place where community activism could flourish. My personal library that includes law books on Indian law was included in order to further both education and community activism.

Instead of duplicating services some other agencies might offer I wanted to find out what services other agencies were already providing and then get the needed resources to my clients. I would do whatever was necessary to help my clients with the obligatory paperwork and other obstacles. Today most grant providers are stressing cooperation and networking amongst non-profit organizations. This is something that not only am I good at organizing, but I truly enjoy this work.

CHAPTER TWO

IN THE BEGINNING

All my life I have heard about Indian Time. Nothing in this life works so independent of rhyme or reason as Indian time. That was the first of many lessons that the project of establishing First Nations Tribal Library and Social Research Center taught me. The tribal organizations that I worked with were generous, but had no interest in real deadlines or timing I might be facing. For instance, it took six months to just get approval to allow phone lines to be installed in my office.

Luke Madrigal is the director of Indian Child and Family, a social service agency for Native Americans. Luke and I had become friends, so I had suggested that it would help us both to have offices in San Bernardino. Although Indian Child and Family services had had offices in town in the past, at the time that I was talking to Luke the agency only had one office in Temecula, which is two counties over. I felt that it

was important to offer as many services to the Urban Indians in San Bernardino as possible. That also included foster care programs, which was a specialty of Indian Child and Family Services.

I had worked with the only agency in San Bernardino that serves Native Americans. They offered some services but most services were limited to only registered Indians. That has always been a problem since so many Native Americans have relocated to cities. Often these people lose their tribal identity and their children may or may not be registered. The Bureau of Indian Affairs conveniently only allows their funding to be spent on who they recognize as being Indian. Despite acceptance by Native American community, the Bureau of Indian Affairs disallows people who are not officially registered. Even though, through the years, it has been acknowledged that some of the older rolls being used by the Bureau of Indian Affairs for identification are in error. The federal law allows the Indian community to include people they deem as Indians.

In 1997 I had the privilege of meeting and becoming friends with the then chair of San Manuel Band of Mission Indians, Henry Duro. I can't express how honored I was to know a man so dedicated to bringing self-reliance to his people. I never spoke to Henry that we were not engaged in how to enhance the struggle of Native Americans in fitting into the foreign system of government that is now over all people in the Americas.

Along with my desire to help Indian Child and Family find an office and my need to have an office to work out of, I asked Henry if Luke and I could have an office at San Manuel. Henry stated that San Manuel had just received an office building from the federal government at the disbanded Norton Air Force Base. The building was handed over to the San Manuel Nation to use to provide social services for Native Americans. Henry said we should make a formal request to the tribe. Luke and I then began a series of meetings and correspondence with the San Manuel Nation. It took over a year to get results from the San Manuel Nation.

There were several important issues facing the San Manuel Nation. This small Nation, consisting of about 70 people, had, in less than ten years gone from abject poverty to owning a large Bingo Palace, supplying them with a lot of benefits and a lot of new troubles. I was just one more person wanting something. The business committee was always nice when Luke and I saw them. However, permission to move into the office they provide took over a year. Several times we would show up at a meeting only to find out the members of the business committee were not even in town. They had been called out of town on business and had left without notifying us that our meeting had been canceled.

Luke at this point told me we should give up. However, I felt it was important for the San Manuel's to be involved because they are the local Native Americans and they should always be considered first. I got a call and was told by the person on the other end of the phone that Luke had cut a deal with a tribal member to get an office without me. That was Luke's right to fight any

way he could. He is a California Indian and I am not. For some California Indians that is an important point. California Indians were the most abused of all Native Americans. During the Gold Rush period of California's history more California Native Americans lost their lives than in any other period of American history. In the 1950's the relocation program initiated by United States government, which was an attempt at forced assimilation as well as bolstering a cheap labor market ended up bringing millions of non-California Native Americans into southern California. This relocation of Native American was a strain on resources. Currently the San Bernardino area is home to more than 125,000 Urban Indians, according to statistics gathered by Arrowhead United Way. The California Indians were pushed into an uncomfortable position of sharing meager and depleted resources with people whom they had little in common.

Although tribal entities are always sensitive about their territory and pride, this competition for

resources increased the problems between the different groups. Having been raised in California it felt like my home, but I knew that most California Indians were tired of all Indians being portrayed as one group. Hollywood and the media only seem to have one image and that is of the plains groups (like the Lakota and Dakota Nations). The true images of California groups are either missing or delegated to "diggers" (a racially charged term, used to refer to a group of unsophisticated people). The Native Americans living in southern California before the arrival of Europeans were living a very idyllic lifestyle due to the mild climate and abundant natural resources. The Europeans, who had a radically different lifestyle, choose to use the pejorative term "Digger" to refer to these peaceful family groups. Knowing this fact, it is hard to accept the derogatory term "Digger." I have given this explanation to point out the position I sometimes find my self in with California Indians. I am an Apache, Choctaw and Cherokee. In other words I am an outsider.

When I found out I was about to be cut out, I called a few friends I had that could have an influence to help me get the office. The committee meeting that made the difference was a closed session, so I will never know the whole story but I am sure that Henry Duro, and a few others were on my side. I got my office.

Laurie Green had become a great friend. Laurie's husband was a San Manuel Indian. I had sold him some American Indian Movement t-shirts. Laurie worked for the then senator Joe Baca, so she gave me a valuable contact to a strong local politician. Laurie also gave me the first office equipment and helped me acquire my business name and license. My husband and I went to a sale at the University of California at Riverside, where we got three bookcases, a table and a filing cabinet.

It took a month to move in all my furniture and log in my books. Elija was a fellow activist from Holland and was raised by a family of librarians. She and I designed a system to keep track of the books. I love my library but I am not a bookkeeping person. The focus was

never to be the books. The books were, however, useful and they did get us a lot of attention. The press and some officials liked my activism but found the library less threatening. I did a lot of press interviews and attended every group meeting I could to spread the news.

Joe Rodriguez in the County supervisors office got us more desks, as I wanted to have computers to use for those using the library. Today the World Wide Web is bringing Native American activism into a more cohesive front. Many local Native Americans from outside California have a difficult time staying in touch with their family on distant reservations. I felt that we could use computers to bridge that gap. Today most reservations, including some of the poorer reservations have a computer at the Nations' headquarters.

It took almost six months to get official approval from the business committee to have phones. They finally said we could have phones when I pointed out Luke was running the office from Temecula. In addition, the fact that my having to borrow the phone at the security desk

was an imposition to our neighbors (the marketing department for San Manuel). I have a temper and after receiving several lectures about the marketing department being on one side of the hall and my offices being on the tribal side, inferring that I was a disruption to marketing, I asked the person annoying me, if it made a difference which side of the hall they were standing on when the tribe fired them. After that I was left alone.

CHAPTER THREE

BUSINESS AS USUAL

By this time I had learned to spend several days a week networking with other agencies. I liked this part because I was able to meet people and sometimes I was the first contact these people have with the Native American community. If you open up to people you would be surprised how much they want to help you, and you will receive your backing. This was important because the office had no financial backing and I had to have my first fund-raiser. Contacts I made at Kiwanis such as Joe Rodriguez, and the gentlemen at the Gents Organization helped me with my first fund-raiser. The Gents are a wonderful club of local men who help the community. They offered their large banquet room for free.

Jamie Alvarez, a community business leader and also a Kiwanis member, offered to cater my Fund-raiser and cover all costs for the food. With the cost so low I thought we had it made. I was wrong. We sold few

tickets and if all my friends were not invited for free it would have been a disaster. Luckily enough tickets sold to pay what cost we had. I decided that all the work, and money we used for the dinner fund-raiser was not worth it so we gave up on that idea.

I had permission for phone lines to be installed around June. It was costing a little over $300.00 a month. At this time I thought I would sell library passes. I sold around 15 at $20.00 each, which helped with the phone bill. Money was a problem. I looked into getting a non-profit status so I might write a grant. Friends of mine who run the Fontana Native American Indian Center had a non-profit. When I learned that it cost a lot to get started, they offered to help me. Fontana Native American Indian Center actually drew up a contract to cover me with their program so I could get started. I also learned that people will check out books, but you cannot get some people to bring books back. The funny part is some of the books not returned were lent to rich people who could have bought all my

books. Humans will always be human. I learned that the people who did not return the books felt the books were not important enough to remember to return.

Two remarkable people, Carol and Ellis Ray, run Fontana Native American Indian Center. They have played very important roles in my life for several years. Carol and Ellis have given me support, answered questions, and some how knew when to give me space. Hard knocks are part of the process. Every time I stumble one of them seems to be close enough to dust me off and cry or laugh with me. They fill a rare spot I value dearly.

In September of 1999 I attended a 5-day class on grant writing. After 5 days I was sure I did not have what it takes to write grants, but I had learned some basics. I now can draw up a budget, and keep enough paper work that a grant writer can help me. I have found a grant writer that is willing to work with me and we are in the process of writing for a grant that looks promising. If you allow handicaps to stop you it becomes a dull life. A handicap is just a challenge you must

answer. I can work with people and find resources but I cannot do math type problems. I was beat a lot as a child and my ability to function well in math. I face that head on and find others who I can trust to help me do that type of task.

Before the end of the first year I had learned a lot about the need to sometimes be formal. I now have an active board, six members strong. I try to surround myself with people that are pro-active, experienced in areas I am weak. I listen to their advice knowing I need them, but have them understand that in the end I am the responsible person. People allowed to show their talents always do their best. A smart leader allows that room, and then gives that talent proper notice. Awards, flowers and gifts are all important.

CHAPTER FOUR

TO EVENT OR NOT TO EVENT

Native Americans, as a community have a problem. At school and in the media Indians have been portrayed as alive and viable only in the distant past. Our children need to have current and correct images. One of the children from Fontana Native American Indian Center had come home crying about this problem. In school she had proudly told her teacher that she was an Indian. Her teacher said something that is unfortunately typical. This uninformed teacher told this child that she was sure everyone had a little Indian blood but all the real Indians died in the last century. This young child experienced what all Native American children face: do real Indians exist outside history? How about the fact that movies only show "feathers and beads" with titles like "The last of ..." or "The Vanishing ...." or many words that say these people are dead.

I was teaching a Japanese lady about Native Americans when she told me she had traveled all over

America. Everywhere she was she had looked for someone who looked like an Indian. In Japan many people wore traditional clothes, but in America she had never seen an Indian. What she didn't understand was that feathers and beads do not define an Indian.

Native America has an image problem; we as a people have vanished from the sight of everyday America. I decided my way to counteract that was to wear clothes that spoke about my ancestry, and be very active in the community so people would understand we are here, today. Since that time I am very careful to openly wear large amounts of silver, sometimes beadwork and oftentimes a medicine bag. I also am very proud and often wear a very expensive pair of custom-made knee length moccasins. Oddly enough, my habit of wearing cultural clothes has gotten me into a great deal of trouble. I have been written up and fired from jobs. Even at California State University San Bernardino a professor felt that it was important to single me out for negative verbal and written sanctions over my wearing of cultural

clothing. This teacher had loudly explained at every opportunity that she too was Native American. Despite her declaring that she was Native American, her attempt at lecturing on Native Americans revealed a distinct lack of actual knowledge or experience. I also acquired a letter from the Brothertown Nation (the Nation that she claimed was her background) that she was not a member.

During the first year First Nations Tribal Library and Social Research Center had several events I am proud of. The first formal event was the East Valley/ San Bernardino Area Diversity Conference. I personally feel that it is important for Native Americans to be seen as alive today. This conference gave me a chance to support a community effort. The conference opened lines of dialog between different ethnic voices in the community. We were co-sponsors and I delivered a speech.

Often I am asked where do you go to meet a real Indian. San Bernardino has had a reservation on the edge of town since the early 1900's. In the 1950's thousands

of Native Americans were either "relocated" by the government or came here for jobs such as the railroad. The official count of the county is only a little over 20 thousand but the United Way paid for a private counting and they got over 120 thousand. Both are probably off because of the problem of how you identify a Native American. There are surely 60,000 plus Native Americans in San Bernardino County. In the past, people would hide their Native American background. Today people seem to invent Native American blood where there is none. One way or the other, we are aware of a large unserved population. The latest release of numbers from the Din'e Nation say that half a million of their registered people live in Southern California and many live in San Bernardino because of the railroad jobs.

I attend the school board meetings for San Bernardino. Again I felt the Native American community needed to be represented. There are usually three of us always there to watch out for our communities: Gill Navarro from Mexican American Political association

(M.A.P.A), John Moore from the National association for the Advancement of Colored People (N.A.A.C.P.), and me for the American Indian Movement (A.I.M.). We have all learned to watch for each other and actually work together. In addition to providing my library for use and offering free services (which were not used by the school district), I was fighting the racist mascot issue. Though the mascot issue is a huge issue for Indians, other communities generally don't understand or care about it. In San Bernardino we made a united effort with M.A.P.A., N.A.A.C.P. and A.I.M. It took a year but now there are no "Indian mascots" in San Bernardino City Unified School District. In addition many of the surrounding school districts are also changing. I never understood how it could be considered an honor to use any human images as mascots, especially when the people say they do not appreciate that "honor." A mascot is a pet; an honor is a raising of status.

My next event was to take a class about the school district's policies on suspension and/or expulsions.

After I took the class I was certified to be an educational advocate for the San Bernardino school system. I placed a sign on my wall at the library advertising my services for free.

The next event was a large community effort. It was a collaborative effort called "Uplift the Community: Empowerment Faire '99." The effort was started and run by Inland Behavioral and Health Services Inc. It was one of the largest and best community efforts I am aware of for our area. Any group that helped people get empowered was invited. There were health groups, a tobacco recovery group, drug and alcohol recovery, local politicians, voter registration, and many other community service organizations.

At this point I was staying busy but still not bringing in any regular source of money so I tried a "Read-a-thon." The idea was to get students at San Bernardino Valley College to acquire sponsors. The sponsors were to pledge a money amount for how many children the student read to during this event. The lady

in charge of the college reading lab was my contact person. There were several meetings to plan this event. However, like earlier attempts we were not successful. I receive a donation from the lady who worked on the project with me and one student collected $15.00.

Sharon Caballero, president of San Bernardino Valley College, and I had become friends. Sharon and I had conversations about the needs of the community. Sharon has the unique ability to challenge people to do work they have insight on, and help them to succeed. Sharon and I began to make plans to help bring the minority communities together. I designed an event to include all under represented communities. Sharon was able to provide a place to have the event. Through her we were able to encourage professors who belonged to under represented groups to be a part of this event. By this time I was learning to be more formal. I formed a committee. We then built a mission statement, sent out invitations and had a very exciting event. The feedback

has been so encouraging that we will make this a yearly event.

In November I was asked to lecture at California State University Northridge. Dr. Nava had heard about First Nations Tribal Library and Social Research Center and ask me to speak at a class. It was a good class and many students asked me to stay after and speak more on Native American issues. I really enjoy lecturing so I hope to increase these events.

I carried supplies and posters for the census. I spoke out at meetings and anywhere I could. It was important to get the community involved. I understand the urban need to count Native Americans as so many of our people avoid any contact with a government that has so disenfranchised the Native American in its system. On the other hand all reservation Native Americans are registered with the government as members of an Indian Nation. They are well documented, I don't understand the government miscounting these people. In the last census all Native Americans with Spanish surnames were counted

as Latino, denying the fact these names were forced on the people and had no basis in Latin America. The government officials we spoke to agreed this happened and promised it would not be repeated.

We closed out the year with a holiday party. It was well attended. The people we had networked with all year dropped by to share food and conversation.

CHAPTER FIVE

AT LAST A RAP-UP

It was a long and good year. I started with no idea where I would find my services valuable. I know there was a huge void of services to Urban Indians and a smaller but important need for reservation based Indians. I had no idea what I needed to have a viable resource center. I have made huge strides in learning the importance of formality and paperwork. First Nations Tribal Library and Social Research Center has a governing board now. It was formed in the later months of the year. We changed our name to First Nations Tribal Education and Resource Center. First Nations is the short and informal version of our name. The board decided that we needed to narrow our focus, improve on what we do well, and not be a lending library. As a library I could get people to borrow books but not return them. After losing several books it was decided that we would continue to have the books available to

the public, but people could not check them out and remove them from the premises.

First Nations also needed to move from San Manuel's office building. Although the San Manuel people had been generous and supplied a free office space, I was only allowed to use the office between the hours of 8a.m. to 5p.m. The people I was hoping to service often times could only use my services evenings and weekends. After months of asking the San Manuel's for additional hours I was told they could not extend the hour of availability for my office.

First Nations Tribal Education and Resource Center is now in the process of relocating and we have submitted a formal request for grants that look promising for us to be fully funded by fall 2000. Narrowing our services was key. In the mean time I am operating from home and when needed from Fontana Native American Indian Center.

APPENDIX A:
FIRST NATIONS TRIBAL LIBRARY
AND SOCIAL RESEARCH CENTER EVENTS

East Valley & San Bernadino Area Diversity Conference

SPONSORS

East Valley Building A Generation
Racism Free Coalition
University of Redlands
Loma Linda U., Office of Vice Pres. for Diversity
The Mayor and City Council, City of Yucaipa
New Hope Missionary Baptist Church
(San Bernadino)
San Bernadino Community College District
Redlands Police Department
Community Advocates for Education Equity / CAEE
Charlie's Angels: Adopt A Bike / Computer
Human Relations Commission, City of Redlands
Redlands - Yucaipa Guidance Clinic Association, Inc.
First Nations Tribal Library & Soc. Research Center
NAACP, San Bernadino Branch
IMPROVE, Business & Communication Association Inc.
Westside Action Group (WAG)
Westside Story Newspaper
The American News
The San Bernadino Community League of Mothers
Latino Impact

PARTICIPATING INSTITUTIONS

Redlands Family YMCA
Redlands East Valley High School
Redlands High School
Department of Education, U. of Redlands
Office of the Mayor & City Council, Cities of:
***Loma Linda, *Redlands, & *Calimesa**

East Valley / San Bernadino Diversity Conference

CRAFTON HILLS COLLEGE
Saturday, April 24TH

Morning

• Registration	8:00 - 8:45 a.m.
• General Session & Keynote Address	9:00 a.m. - 10:30
° Small Group Workshops	10:30 - 11:30
° Closing Session	11:30 - 12:30
• Lunch & Individual Interaction	12:45 - 1:45 p.m.

Afternoon

• Registration / Sign-In & Receive Packets	1:30 - 2:00 p.m.
• General Session & Keynote Address	2:00 - 3:30 p.m.
• Small Group Workshops	3:30 - 4:30 p.m.
• Closing Session	4:30 - 5:00 p.m.

DIRECTIONS TO CRAFTON HILLS COLLEGE: I-10 East pass Redlands to Yucaipa; take the Yucaipa / Crafton Hills College Exit; turn left over the freeway (you are on Yucaipa Blvd.); continue on Yucaipa Blvd. for 2 miles; turn left at the signal - Canyon Hill (there is a Crafton Hills College sign on the right); go up the hill to the entrance sign to Crafton HIlls College. Handicapped Parking in lots D, E, F & G.

LOMA LINDA UNIVERSITY
(Randall Visitors Center)
Thursday, April 29TH

• General Session & Keynote Address	6:00 - 7:00 p.m.
• Small Group Sessions	7:00 - 8:00 p.m.
• Closing General Session	8:00 - 8:30 p.m.

DIRECTIONS: I-10 East From San Bernadino to the Anderson / Tippecanoe Exit; turn South on Anderson. Pass Loma Linda Academy & Stewart Street. Turn right at the next signal. Randall Visitors Center is on the right hand corner. Beyond Randall Visitors Center in the Library.

•Certificates of attendance are available upon request.

Keynote Speakers

Brenda Campbell- Five years as a classroom teacher; Eight years as a principal; Director of the S.B. County Schools School Leadership Center since 1993; served for four years on the State Chapter 1 Comm., served on the Ex. Bd. of the Calif. Institute of School Improvement (CISI); received 134,000 votes as a candidate for the SBCUSS; recipient of the U. of Redlands' 1999 Outstanding Alumni Award; a highly sought after speaker.

Dr. Elsa Valdez-Currently Prof. of Sociology, Calif. State U. of San Bernadino & a member of the San Bernadino City Unified School District School Board; former president of the San Bernadino MAPA Chapter; numerous articles and publications; a highly sought after speaker.

Jim Bueermann-Chief of Police, City of Redlands; Original Ch., East Valley Building A Generation - Cities That Care.

Diversity Conference
Planning Committee Members

Dr. Amos Isaac, Chairman

Ms. Yolanda Contrares

Captain Tom Fitzmaurice

Mr. John Lujan

Mr Ray Quinto

Ms. Delores Coles

Ms. Vickie Jackson

Dr. Leslie N. Pollard

Mr. Mark Shepherd

Ms. Billie Daniels

Dr. Randall Lindsey

Ms. Pamelalee Bailey-Schimizu

Ms. Mary Trost

REDLANDS
POLICE

THE SAN BERNARDINO AMERICAN

IMPROVE
Business & Community Association Inc.

WESTSIDE STORY
News Of The Empire

East Valley & San Bernardino Area Diversity Conference

RACISM FREE COALITION

Saturday, April 24TH

CRAFTON HILLS COMMUNITY COLLEGE

&

Thursday, April 29TH

LOMA LINDA UNIVERSITY

(Randall Visitors Center)

20 COSPONSORS

&

7 PARTICIPATING ORGANIZATIONS / INSTITUTIONS

3 Keynote Addresses
18 Small Group Sessions
5 + 5 + 5 Exchanges

East Valley / San Bernardino Area Diversity Conference

Saturday, April 24, 1999: Crafton Hills College

Morning Session

Moderator: Mr. Wallace Allen

Synthesizers: Dr. Randall Lindsey Dr. Clarence Goodwin
Ms. Beverly Jones Wright Dr. Patricia Little Dr. Cecelia Juliagay

Small Group Workshops

Workshop A:

Native Americans in the Curricula of Public and Normal Schools:Formuli for Genocide Or a Valuing of Native American History, Culture, and Diversity. An examination of mission statements, preparation of school personnel, and curricula offerings.

Presenter:
Ms. Pamelalee Bailey-Shimizu, Ex. Director of the American Indian Movement San Bernardino Chapter & Founder & Ex. Director of First Nations Tribal Library and Social Research Center.

Workshop B:

How to Find Agencies & Programs That Can Help Me: Connecting with Community Resources. What agencies and services are available & how to access them.

Bilingual: Spanish / English presenters

Presenters:
Ms. Martha Amaya & Ms Henrietta Garcia: United Way of the East Valley

Workshop C:

Assisting Students with Practical Help, Emotional Support and Encouragement. EACH ONE, TEACH ONE Learn how to establish a mentorship program.

Presenter
Mr Lawrence L. Daniels, Mentor Program Coordinator

Workshop D:

Scholarships & Mentoring Programs

Presenter:
Ms. Clarice Richards, Scholarship Chair, New Hope Missionary Baptist Church

Inland Behavioral and Health Services Inc
1963 North 'E' Street
San Bernardino, California 92405

Up-Lift the Community "Empowerment Faire"

October 5, 1999

Pamela Lee Bailey-Shimizu
1st Nations Tribal Library
1482 E. Enterprise Blvd., Suite 466
San Bernardino, CA 92408

Dear Pam:

The staff and Board of Directors of Inland Behavioral and Health Services, Inc. would like to extend our heartfelt thanks to you for working and participating in the Uplift The Community First Annual "Empowerment Faire" a wonderful achievement! People like you make all the difference in an events success.

As we prepare for next year, remember that with your continued support and effort, the year 2000 faire will be even more successful. See you next year!

Sincerely,

Temetry A. Lindsey
CEO/President

Edward L. Williams, LCSW
Vice-President of Treatment

Linda Hart
Event Coordinator

Serving the health and welfare needs of our communities

CERTIFICATE OF APPRECIATION

This certificate is awarded to

Pamela Lee Bailey-Shimizu

in recognition of valuable contributions to

Up Lift The Community: Empowerment Faire '99

Edward L. Williams, LCSW, Vice-President — Date: 10-5-99

Linda Hart, Event Coordinator — Date: 10-5-99

Inland Behavioral and Health Services, Inc.

First Nations Tribal Library and Social Research Center's Annual Read-a-thon October 18-29, 1999

In an attempt to promote quality information and have fun with children, First Nations Tribal Library is promoting a read-a-thon. We are challenging students at Valley College to go into their own communities and find places where they can read Native American stories to children. We will provide the stories and any assistance or information needed. Each person signing up to participate in this read-a-thon will be requested to find sponsorship. Sponsorship means that a person offers to pay a certain amount of money per child read to. For example: A "reader" has five neighbors, each of whom agrees to sponsor the reader $1.00 per child read to. The "reader" then goes to a local school, church, community center, park, etc. where he/she reads to the children. Each child initials a form, and then the "reader" counts the number of children. He/she goes back to the five neighbors and reports the number of children read to. If 10 children were read to, the "reader" would collect $10.00 from each of the five neighbors, for a total of $50.00. The college student will be totally responsible for keeping track of the number of children read to, and the collection of sponsorship funds.

The student who reads to the most children will receive a book grant (dollar amount to be determined based on the success of this activity) for the next semester, and there will be a book grant to the student who raises the most money. Money collected should be turned in to the First Nations Tribal Library or the SBVC Reading Lab by November 1, 1999.

First nations Tribal Library will be happy to assist wherever needed. Students can check out a book from the SBVC Reading Lab. Students must present a valid SBVC Library Card, and will be fully responsible for the cost of the book if it is not returned in good condition. Books can be checked out for a period of no more than three days.

Each participant will receive a First Nations Tribal Library Card when funds are submitted.

First Nations Tribal Library and Social Research Center's Annual Read-a-thon October 18-29, 1999 Sponsor Sheet

Sponsor	Dollar amount per child read to	Total	Collected	Turned into library
Dana Corbin	Lump Sum	$15.00	$15.00	

First Nations Tribal Library and Social Research Center's Annual Read-a-thon October 18-29, 1999

Child Sign-In Sheet

Participant Name Diana Cerdero

Title of Book Crow & Hawk & Between Earth & Sky & Hidden Spirits

All children initial below:

Angelo Cervantes	8 yrs
Alec Cervantes	6 yrs
Isaac Chagolla	4 yrs
Heavenly Chagolla	2 yrs

Class of 21 Pre-school students 4 year olds

San Bernardino Valley College

Office of the President

November 4, 1999

Ms. Pam Bailey-Shimizu
First Nations Library and
American Indian Movement
1482 Enterprise Avenue, Bldg. 466
San Bernardino, CA 92408

Dear Pam:

Thank you for your attendance to the First Educational Summit on October 29, 1999. The discussion was very enlightening and informative. The Summit reconfirms my belief that we need to continue discussions to increase our understanding of race, gender, class and sexual orientation to make the educational system a successful journey for all children.

Without your support, the Educational Summit would not have occurred. In addition, I would like to thank the First Nations Library and Social Research Center, and the American Indian Movement of San Bernardino County for their support of the Educational Summit. I would also like to extend a special thanks to Senator Joe Baca's Office and Assemblyman John Longville's office for their generous donation of scholarships to the Educational Summit.

Attached is a list of participants at the Educational Summit, as well as notes taken at the event. To continue the momentum of the Summit, we are instituting a monthly meeting to move the discussions forward. We would like to schedule our first meeting for the end of November. If you could please call Pam Bailey-Shimizu, First Nations Tribal Library at 382-2204 or 888-0228, and leave a message when you are available to meet between November 15-30 anytime from 8:00 a.m. to 5:00 p.m., Monday through Friday.

Again, thank you for you commitment to explore ways to increase the presence of underrepresented people in our schools' curriculum.

Sincerely,

Sharon S. Caballero

Sharon S. Caballero, Ed.D.
President

SBVC

701 South Mount Vernon Avenue • San Bernardino, California 92410 • (909) 888-6511
San Bernardino Community College District

October 29, 1999 Educational Summit Attendance List

First Name	Last Name	Organization	Address	Phone
Leonard	Lopez	SB Valley College	701 S. Mt. Vernon Ave San Bernardino, CA 92410	909-888-6511 X 1563
Steven	McGrew	CAPE Rainbow Council	279 E 34th Street San Bernardino, CA 92404	909-886-8533
Gil	Navarro	California Parents Assoc.	985 W. Kendall Drive San Bernardino, CA 92407 PMB357	909-787-6027
Laverne	Pine	FNAIC	365 W. Grove Rialto, CA 92376-4907	909-820-6365
Beverly	Powell	SB Community College Dist	701 S. Mt. Vernon Ave San Bernardino, CA 92410	909-307-6742
Ruby	Ramirez	Senator Joe Baca's Office	201 N. "E" Street San Bernardino, CA 92401	909-885-2222
Carol	Ray	FNAIC-SB/R NACC	PO Box 1258 Fontana, CA 92334-1258	909-823-6150
Ellis	Ray	A101 & FNAIC	PO Box 1258 Fontana, CA 92334-1258	909-823-6150
Judith	Reuther	SBCSS	601 North E Street San Bernardino, CA 92410	909-387-4433
Sara	Rubalcava	Liberia Del Pueblo	972 N. Mt. Vernon Ave San Bernardino, CA 92411	909-888-1800
Bill	Runyan	Retired Educator, Columnist	17319 Reed Street Fontana, CA 92336	909-823-4578
Sue	Runyan		17319 Reed Street Fontana, CA 92336	909-823-4578
Henry James	Vasquez	SB City Schools IMISA	660 S. 5th Street Colton, CA 92324-3303	909-825-0208
Joe	Virata	UC Riverside Asian Pacific Student Prog	104 Costo Hall Riverside, CA 92521	909-787-7272
Renea	Wickman	Holistic Healing for Youth	1405 Mills Avenue Redlands, CA 92373	909-307-6246
Nancy	Wolfe	Indian Child & Family Services	28441 Rancho Calif Rd, Suite J Temecula, CA 92590-3618	909-676-8832 X 19

Educational Advisory Committee

10/1/99

Carol Clay		
Ellis RAY	AOIC SB. County	823-6150 ext PTRINCHAKAM
Julia L. Bogany		909-885-4317
[illegible]		
[illegible]		
[illegible]	

Rough Draft

Educational Summit Mission Statement

The_____ Mission is to meet and discuss issues and problems that any under-represented group feels about how they are portrayed in education.

The_____began on August 20th, 1999

KEY OBJECTIVES

TO PROMOTE curricular changes in educational institutions that will depict positive under represented groups contributions to the fabric of local, state, national and world history.

TO PROMOTE programs in educational institutions with linkage to industry that will encourage under-represented groups to pursue higher educational goals, professional or technical careers.

TO IDENTIFY exemplary multiethnic and minority programs at the elementary, secondary and university levels which can be replicated in local educational agencies.

TO IDENTIFY exemplary multiethnic and minority curricular materials, r3sources or instructional units for implementation and use at the elementary, secondary and university levels.

TO IDENTIFY and link with local and area minority persons who may serve as appropriate role models for multiethnic and other minority persons.

Educational Summit
Planning Meeting # 7

Friday, October 8th, 1999 - 11A.M.
First Nations Library and Social Research Center
San Manuel Business Office

M I N U T E S

The seventh planning committee meeting of the Educational Summit was held on Friday. October 8th. 1999 at the San Manuel Business and marketing office. The meeting was called to order by Chairperson Pan Bailey-Shimizu at 11:15a.m.

Present were: Nancy L. Wolfe. Julia L. Bogney. Anthony Castro. Ellis Ray. Pamelalee Bailey-Shimuzu. Carol Ray and LaVern Pine.

Minutes of the October 1st meeting were accepted as presented.

OLD BUSINESS:

Handouts - AGENDA

8:30 to 9a.m.	Registration (Continental Breakfast)
9:00 to 9:15 a.m.	Welcome by Sharon Cabrillo. Valley College President
9:15 to 9:30a.m.	Educational Summit Introduction - Pam bailey-Shimizu
9:30 to 10a.m.	Key Note Speaker - Professor Ed Gomez
10 to 12 p.m.	Individual Group Workshops
Noon	Lunch
1 to 2:30 pm	Summit Outcomes and Goals

LETTER FROM FIRST NATIONS LIBRARY
DRAFT OF MISSION STATEMENT
ROLE MODELS (Tease Sheet)
NOTE FROM INDIAN FATHER TO CHILD
MAP OF CALIFORNIA TRIBES
1964 CIVIL RIGHTS PROCLIMATION
OTHER ?

MISSION STATEMENT DRAFT - see Exhibit 'A'

Thank you to San Manuel Marketing Department for donation of 100 bags, pens and notepads.
Thank you to Fontana Native American Indian Center for donation of 100 handout folders.

A REAL BIG THANK YOU TO *ANTHONY CASTRO* FOR SUPPLYING THIS MEETINGS REFRESHMENTS.

ADJOURNEMNT: Meeting adjourned at 1:30p.m

Respectfully Submitted.

Carol Ray

Education Summit October 29, 1999 - RSVP List (Updated 10/18/99)

Last	First	Company	Yes	No	Comments
Abdulmumin	Alfred	UCR			
Adams	Willie & Evon	American Indian Church			Senator
Baca	Joe		1		
Baguley	Linda	Title IX Indian Education	1		
Bailey-Shimizu	Pam	First Nations Library & American Ind Mvmt			
Beller	Fred	WestEd			Senator
Boxer	Barbara				
Brown	Marta	Candidate 42nd District			Senator
Brutle	Jim		1		
Caballero	Sharon	SBVC			
Castro	Anthony	SB Sexual Assault Services		1	
Caulderon	Maunce	Arrowhead Credit Union			
Cerda	Tony	Costanon Rumsen Ohlone			
Chacon	Martha	Elder San Manuel	1		Is sending Judie Reuther
Ciaun	Robert	Career Training & Supp Svs			
Cortez	Carlos	UCR			
Dalton, Ph.D	Henry	Publisher, Retired Educator			Superintendent
Delgado	Anthony	SB Cty Schhol			
Drake	Barbara	SB Title IX Indian Ed			
Duro	Henry	San Manuel Band of Indians			Senator
Feinstein	Diane		1		May be late
Fenelon Ph.D.	James	CSUSB			Superintendent
Fischer	Herb	SB Cty Schhol			
Frazier	Joanne	Press Enterprise	1		
Garrett	Connie	U of C Cooperative Ext.			
Gomez	Vincent	Educator			
Grant	Dorothy	Retired Educator & Community Activist			
Hart	Linda	Inland Behavioral Sci & NAACP			
Hawkins	Maggie	Lesbian, Gay & Bisexual & Transgender Rch Ctr			
Isaac, Ph.D	Amos				
Jacobo	Robert & Eva	Azteca Traditional Research			
Jiminez	Eric & Pam	Traditional Leader	1		880-1921
Jones	Bettie	Educator			
Julagay, Ph.D.	Cecelic	CSUSB			
Knapp	Robert John	Spiritual Leader			
Leroy	Lynn	San Manuel Tribal Council			Representative
Lewis	Jerry				
Little	Dr. Patty	CSUSB			Pam said may pur tbl 10/5
Longville	John	Assemblyman		1	
Marx	Dr. Marcia	CSUSB			
Navarro	Gil	MOPA & CA Parents Assoc			
Oyote	Rep				
Pezant	Lita	Westside Story			
Pine	Laverne	AIOIC Opr Comm Member FNAIC Adv			
Pine, Ph.D	Charles	Psychologist			Board Mbr
Powell	Beverly	SBVC			
Ramos	James	San Manuel Cultural	1		
Ray	Ellis	FNAIC & AIOIC of SB Cty	1		
Ray	Carol	FNAIC & SB/Riv Counties NACC	1		
Reuther	Judie	Career Training & Supp Svs		1	
Rivera	Thom	CSUSB			
Roberts	Jo Ann	Phoenix Bookstore	2		
Runyan	Bill & Sue	Retired Educator, Columnist			
Sisquoc	Lorene	Curator, Sherman Museum			
Sisto	Earl	NASP & EOP			
Slaughter	Roger	Native Traditionalist			Assemblywoman
Soto	Neil				
Stalley	Pam	Connection Leadership Project			
Texeria	Dr	CSUSB			
Thompson	Sherry & Lloyd	Native Resources			
Thomson	Sarah	Educator			
Torres	Nana	CSUSB			
Valdez	Elsa	CSUSB			
Vanquez	Henry	Educator	1		
Virata	Joe	UCR			
Weber	Cindra	SB Title IX Indian Ed	1		NOTE Name/Org Change
Wickman	Renea	Wholistic Healing for Youth			
Wolfe	Nancy	Indian Child & Fam Svs			
		Totals	14	3	

CITY OF
San Bernardino

OFFICE OF THE MAYOR
JUDITH VALLES
MAYOR

November 23, 1999

Dear Community Leader,

The purpose of this letter is to request your participation in an organizational meeting for the CENSUS 2000 "Complete Count Committee" within the City of San Bernardino. The meeting will take place at 10:00 a.m. on December14, 1999 at 300 N. "D" St., San Bernardino. The Meeting will be held in the Council Chambers located in the Lobby. The meeting will last approximately 2 hours.

I, along with the City of San Bernardino Common Council and many other public officials are intensely concerned that every resident in our City be counted. Millions of dollars and many essential programs could be jeopardized should an "undercount" occur as in previous census.

As a community leader who daily interacts with many of our City's residents, your knowledge and organizational skills are urgently needed in this undertaking to ensure a complete count of our City's entire population. Representatives from the Bureau of the Census will be on hand to provide information and respond to questions.

If you have any questions or need any additional information, please contact Debra Daniel, Community Liaison at 384-5133. Please RSVP by December 3,1999.

Sincerely,

Judith Valles
Mayor

JV:dld

300 NORTH "D" STREET SAN BERNARDINO, CALIFORNIA 92418-0001
(909) 384-5133 • FAX-(909) 384-5087

Happy Holiday's

First Nations Tribal Library and Social Research Center

Would like the opportunity
to wish you in person a happy holiday!
Please come enjoy the company of others in our community -

Friday Dec.10th 12noon to 5pm

P.S. if you are busy we wish you to know you will be missed, but we understand and will keep you in our thoughts for a very good New Millennium!!!!!!!!!!

RSVP Pam -382-2204 or Tribal Building 466 (Norton)

First Event with San Manuel Nation

Pamelalee Bailey-Shimizu
Exc. Director of
A.I.M. of San Bernardino Area
25220 E. 17th st.
San Bernardino, Ca. 92404
909-888-0228
aimindee@gte.net

Dear Henry Duro;

I am so excited to announce that Four Moons Pow-Wow has been moved to Cal State San Bernardino this year. I have been working on having an educational affair at Cal State for a long time now. A.I.M. is an official organization on campus and as such we would like to have a educational conference before the Pow-Wow each day. This year we would like to have Saturday morning we will be inviting Bird Singers, and a demo of Pinion players, through the help of Luke Madigral and a speach on the history of gamming amoung California Natives by Pamelalee Bailey- Shimizu a Native American Sociologist. On Sunday morning, we were hoping to invite musicians in to celebrate Native American music. We have a small organization with no funds so I am writing you for help in funding. The following is what we are guessing the cost to be please assist in any or all money needed. We understand you are flooded with request and would not bother you if it was not important to the local Native community.

Bird Singers	$500.00
Pinion demo	$200.00
Tribu	$500.00
Native Flutes (2 or more)	$300.00
Lecturer and M.C. at both events	$500.00
Dinner for Dancers and participants Sat. night at CSUSB dinning hall	$1,500.00

We would like any input or ideas you may have to improve our event as you are important to us. Thank you for your time and I hope to hear soon.

Pamelalee Bailey-Shimizu

APPENDIX B:

CERTIFICATE FOR TRAINING PROGRAMS

CHILDREN'S NETWORK

385 North Arrowhead Avenue, Second Floor • San Bernardino, CA 92415-0049
(909) 387-8966 • Fax (909) 387-4656

COUNTY OF SAN BERNARDINO

KENT PAXTON
Network Officer

July 27, 1999

Pamelalee Bailey-Shimizu
First Nations Tribal Library
and Social Research Center
1482 E. Enterprise, Bldg 466
San Bernardino, CA 92408

Dear Mrs. Bailey-Shimizu:

On July 23, 1999 WE MADE HISTORY !!!! The San Bernardino Unified School District was the first in San Bernardino County to train community residents to be advocates for students who are a potential candidate for suspension and/or expulsion. You were there!!!!

On the behalf of San Bernardino County Children's Network – imPACCT Program, San Bernardino Families for Youth Development Coalition and residents of the San Bernardino Unified School District, I would like to say **Thank You.** Because of your efforts students and families will be empowered while going through the process of suspension and/or expulsion.

You will receive a certificate of completion from the San Bernardino Unified School District. As stated at the training, your name and telephone number will be placed on the list of Volunteer Community Youth Advocates. This list will be distributed to parents of student who are a potential candidate for suspension and/or expulsion.

If you have any questions or concerns please contact Mr. Cardona at San Bernardino Unified School District at (909) 381-1293 or Robin McCall at San Bernardino County Children's Network-imPACCT Program at (909) 387-5361.

Together we shall develop our youth, families and community to their fullest potential.

Again **THANK YOU!**

Sincerely,
R. McCall
Robin McCall, Community Organizer

Certificate of Achievement

awarded to:

Pamelalee Bailey-Shimizu

Suspensions & Expulsions Conference

July 23, 1999

Date

San Bernardino City Unified School District

Student Services Division

Signed

THE GRANTSMANSHIP CENTER

This is to certify that

Pamelalee Bailey-Shimizu

successfully completed the five-day
Grantsmanship Training Program
in Palm Springs, CA
September 13-17, 1999

Norton J. Kiritz, President

APPENDIX C:

LETTERS TO THE COMMUNITY

Letter 1

From the desk of
Pamalalee Bailey-Shimuzu
First Nations Tribal Library and Social Research Center
1482 East Enterprise Dr. Bldg. #466
San Bernardino, CA. 92408-0161

First Nations Tribal Library and Social Research Center was started in January of 1999 with the concept that people were interested in Native America, but did not understand where to go for information. So the library is a place where books have been researched in an attempt to make sure the material is factual. We have books written by Native Americans and about Native America. We also serve as a center for community activism. First Nations has an emphasis on assisting in education whether it is for students researching specific information or providing lecture and further information to teachers.

First Nations Tribal Library is looking forward to a donation of five computers from Southern California Gas. With these computers, we hope to be able to teach people how to research tribal information and provide a place where tribal people can keep contact with there own reservations. We also will be giving free computer lessons to parties interested in Native America on the Internet.

First Nations Tribal Library is also a cultural center. In an effort to provide quality information we have decided to give classes. Our first classes will be on Native American Music, using flute and hand drum. We also are starting a beading society and beading classes. In general, we attempt to fill the gaps in our community and provide information needed about services in the Native community.

We serve the Native American community and the non-natives who are interested in learning about Native America. The only requirement for access to privileges at the library is a $20.00 annual fee, for a library card. With that card, not only do you have free access to classes, but also you can check out books, music tapes and CDs, and a few videos. We encourage you to come, and if you don't have $20.00, other arrangements can be made.

Letter 2

First Nations Tribal Library and Social Research Center

1482 East Enterprise Dr. Bldg. #466

San Bernardino, Ca. 92408-0161

Phone 909-382-2204 fax 909-888-8840

We are pleased to offer a library dedicated to Native American (The indigenous people of all the Americas) literature, information and current issues.

The Center is not financed by any federal, state, or local organization, we rely on partnerships with the community.

We are extremely proud to announce the opening of First Nations Tribal Library and Social Research Center. This center will serve to assist individuals in locating good literature about Native America. Pam, the director is a community activist, and sociologist.

The library has plans to expand the collection of Native American children's books and language books and tapes. We expect to offer a selection of music tapes, CD's and videos. (The selection is compact at this time).

Library memberships are available for only$20. per year, that enables the card holder to check out a maximum of two books. or one tape or video for two weeks. Plans are under way to offer Native newspapers for sale.

San Manuel has graciously allowed us to occupy space in their building "466" at the old Norton AFB. We are not funded! We cordially invite our neighbors to lend their support through memberships and in kind gifts.

For those desirous of visiting the library please call for an appointment, the staff is often in the field. BEEPER # 909-517-0190

We are planning to add Native American music lessons, beading classes and society and computer lessons free to all members in our Fall schedule. Hurry and get your membership and be sure to sign up for our classes.

APPENDIX D:

COLLABORATION AND OPERATIONAL AGREEMENTS

Fontana Native American Indian Center, Inc.
PO Box 1258. Fontana, CA. 92334-1258 909-823-6150
fax: 909-823-6150 e-mail: fnaic@aol.com

COLLABORATION/OPERATIONAL AGREEMENT

This Collaboration/Operational Agreement stands as evidence that - the Fontana Native American Indian Center, Inc.(FNAIC) and the First Nations Library and Social Research Center (FNLSRC) intend to work together toward the mutual goal of providing maximum available assistance for American Indians and other clients who reside in San Bernardino County. Both agencies believe that implementation of this collaborative as described herein will further this goal. To this end, each agency agrees to participate by coordinating/providing the following services:

FNAIC will closely coordinate with the collaborating agency named herein, by providing:

- Fiscal Receivership for the First Nations Library and Social Research Center under the Direction of Pamela Bailey-Shimizu, and
- First Nations Library and Social Research Center will submit to FNAIC, accurate financial records on a timely basis, and
- At least one FNAIC Board of Directors member will sit on the Board of Directors of the First Nations Library and Social Research Center, and
- Each agency agrees to share information and resources in order to impact the quality of services provided.; and
- Each agency will provide the others brochures and hotline telephone numbers, hotline cards, and other materials to be distributed to their respective clients; and
- Each agency agrees to refer to the other for appropriate services; and
- Each agency agrees to provide staff training opportunities when requested; and
- Each agency agrees to participate in community awareness programs and other networking meetings.

The undersigned, as authorized representatives of the First Nations Library and Social Research Center and the Fontana Native American Indian Center, Inc. do hereby approve this document.

For FNLSRC Pamela Bailey Shimizu — For FNAIC Rex G. Ray

Signature — Signature

10-22-99 — 10-22-1999

Date — Date

Together We Are Making A Difference

APPENDIX E:

MINUTES OF BOARD MEETINGS

Board Meeting 1

1st Nation's Tribal Library - 10/22/99
1st Board meeting + Social Research Center

- Discussion w/ marta Brown - Educational Summit meeting and educational reform goals.
  - Library as resource to school system
  - modern / contemporary Heros -

- marta Brown - Summary of Experience in Education
  - UCI2 - Student Affirmative Action Program Development for Early Preparation
  - History teacher
  - Sister - Crafton Hills Dean of Ed.
  - Congressional Offici - Influenced Education
  - New focus on math + Science Development

- Ellis Ray - Discussion of educational problems in Fontana School System
  - Elimination of "Feel Good" programs -

Carol Ray - Voc. Ed Program - Lavern Pine's Design

Pam Bailey - Summary of 1st Nation's Library
1) motivation to promote learning as young people have resources to learn about selves.
2) offer good solid information + access to culturally appropriate literature
3) Goal to offer resources to the community Info + retrival Database
4) Community Activism

Focus of Growth for Library -

1) Expand Resources (materials Development)
curriculum Development - Tools to be used in the educational process - Indian music
- Beading / leather work -
- Class in a box - Tactile materials

2) Current Status -
- 5 computers -
- DeVris - will provide programs for new computers - will come in next week.
- Internet access to community members w/out of state - distant reservations

Download info re: tribes

3) Resource for Foster Children
Access heritage information re: tribes
family trees

4) State - CSUSB
Highest # of grads per yr. = 5

Need for -
- Mentoring / Support / outreach to Indian Students
- Need to open cultural perspective in coursework

5) Erik Riggs - Science Program for Indians - UCR
Fee Student Referrals to this program

Membership of Board members - Potential Board members (?)

Henry Dure - Not in attendance
Hopeful that he will participate in future

- Barbara Drake - video tape presentation - Traditional & Modern Tools
Marie Alto - Herbal Demonstrations -

- Potential to market educational packets

Grantsmanship -

Will be umbrella under Curci + Ellis Rey's Organization
Fontana Native American Indian Center, Inc.

Other Needs:
- Parenting Hotline -
- Fill gaps in services -

Ellis Ray - Documentary on S.B. County Tribes -

Fundraiser - Summary - Readathon

- Pam Bailey - Shimizu - Hoping to draw salary of $3,000 per month once funding comes in

- Have begun Readathon efforts - will be carried 3 wks.

- Tecl. Krug - CSUSB - (Potential Resources) Tutoring program. Financial Aid Package

- Jr. College Focus - SCC students involved in Readathon.
  - Valley
  - Crafton Hills
  - Result of funding - Scholarship Fund

- Child Development Curriculum - S.B. Judy White - City of Readers

- Hope to expand office hrs at San Manuel - 12-7pm

- Vicki Caminada - might be great for elders - Vickie Cum - Communication - Potential

- new Computers - (5) Need (5) CD Rom Drives
  - Pentium Computers
  - 16 mg RAM -

Riverside Computer Store - Net work (?)
Arlington / Van Buren Green Sheet Entry

1st Nations Tribal Library
~~INDIAN CHILD AND FAMILY SERVICES~~

Board meeting 10/22/99
~~VISITORS LOG~~

Please sign in:

Name	Address	City	Phone
Nancy Wolfe	ICFS 28441 Rancho Calif Rd. Suite J	Temecula, CA 92590	(909) 676-8837
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
Marta M. Brown	873 Bernard Way	SB 92404	(909) 883-1913
Pam Bailey-Chimi	3475220 E17	SB 92404	909 [illegible]

Board Meeting 2

1/26/00

Board meeting - 1st Nation's Tribal Library
#2

- call to Order

- Ms. Bailey Shimizu -
  Problems getting books returned to Library - will create restrictions for book use
  Rename 1st Nation's Tribal Ed. + Resource Center
  Functions of New Center: Change Name + Focus of Center
  - Parenting Classes - Traditional (will look for grant resources)
  - Parent Support Hotline New mothers Target Population
  - computer Resources
  - Tutoring
  - In Library Use of Books
  - March 1st - Goal for move to another
    Reasons - Cannot get extended hours for use + access to library
  - New Location - Fontana
    - San Bernardino
    - may leave this office partially open
  - Generate letter to the tribe re: plans for library
  - memberships - Approx. 15 memberships sold will be honored

- Ellis Ray - Sierra Location - Fontana
  Need commitment about move to Fontana or decline resource.
  - Ideas re: resources for location for library
    - County to County Support
    - Downtown Location
    - Old Santa Fe Station (?)
    - Fontana - Carol + Ellis Ray's' Center

Funding Resources
1) Grants
2) Tobacco $
3) Childrens' Network - Donna Beverage (?)

1/26/00 (2)
1st Nations' Board meeting

- Continue Library or Resource Center (?)
- What Location (?)
- ms. Bailey Shimizu - Request for Discussion of the Preceeding Issues.

Ellis Ray - Proposed various locations - Approx 1 yr. delay in funding on grants. Cal Works Grant - Donna Beverage

Carol Ray - Consult w/ the County to assess areas of need for parenting resources -
- Dorthy Graham (?) School Resources for Funding (?)

Laverne Pine - Library - Small needs 1 location only
Resource Centers - Various locations possible

Pam Bailey - Parenting Classes - Hire someone to teach classes (?)

Education Grant -
- Skills - Hx of Indians -
- Tutoring w/ college work
- Reading - Not preferred
- man Hotline
- Lecture Services (Sovereignty Lecture)

Goal -
- Focus on Grant Resources -
- Consult w/ Michelle Curley - Bill Gates
- Private Orgs.

Move Location Decision -

Ellis Ray
- Back Room - Library + Computer Network
- Computers need to be programed - No operating system on them
- Zero net - 4 programs to make programs y2k compliant
- Need word processing programs - Network server (?)
- Need to make modifications for handicapped access to office bldg.

Pams' Hours for Office
10-11am to 2pm - Not Tuesday
MWF 5pm→
Tues/Thurs - Closed

1/26/00 (3)
1st Nations
Board meeting

- Laverne + Carol to be contracted for grant writing services.

Proposal
Offer → Pam Bailey Shimizu -

- Office Duty in return for Collaboration.
- Written agreement - Proposed to Board of Indian Center Fontana -
- Define mutually collaborative agreement.
- Richard Pam + Maury will pack library to move -
- Discuss w/ Indian Center Board their terms for agreement
- Close 1st Natims' Library + Reopen as 1st Nations Tribal Resource Center -
- Next meeting - 2 weeks - Feb 9th - Noon -

- meeting Closed -

Gary "Wan Eagle" Bromley - using ICFS for "Adoption" services

Board Meeting Attendance
1/26/00

Julia L. Bogany
Carol Ray
Ellis Ray
Lathern Pine
Richard Iron Elk
Paulette Bailey-Shumiya
Nancy L. Wagner

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ABSTRACT

A double blind study in which we evaluated the concentrations of the following five specific metabolites; 3-hydroxykynurenine (3OHK), 5-hydroxytryptophan (5HTP), 5-hydroxytryptamine (serotonin or 5HT), 5-hydroxyindoleacetic acid (5HIAA) and Homovanillic acid (HVA), in cerebrospinal fluid (CSF) taken from the third ventricle of 23 living Parkinson's patients during postero-ventral pallidotomy (PVP) procedure. The patients were standardized by: preoperative selection to undergo PVP, all had severe gait disorders, were on Sinemet at the time of the operation, were in the age range 60-78 yrs old (66.8 Ave.), and had suffered from the disease for more than 6 years. (Average 10.8 yrs). Patients was randomly assigned a number, and had their symptoms scored and recorded using the Unified Parkinson's Disease Rating Scale (UPDRS) by a practicing neurosurgeon prior to the pallidotomy procedure. The concentrations obtained by High Performance Liquid Chromatography with Electrochemical detection (HPLC-EC) were compared between the two subtypes of Parkinson's disease (A and B) with each patient acting as their own control.

The concentrations 5HTP were dramatically elevated, and 5-HT concentrations were significantly decreased in type B PD patients as compared to type A PD patients.

We propose a mechanism based upon the data elucidated and extensive literature review, suggesting a decarboxylase deficiency may cause the accumulation of 5HTP which may be converted to a neurotoxic quinone, which would then speed up the disease progression (characteristic of type B PD) while decreasing serotonin levels, thus disinhibiting the STN and increasing the vGPi resulting in characteristic hypokinetic symptoms of type B PD namely; postural instability, gait freezing, and akinesia.

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CHAPTER ONE

INTRODUCTION

Parkinson's disease (PD), is typically classified as a neurodegenerative motor disorder characterized by the presence of three of the four symptoms of resting tremor (tremor which abates with volitional movement), bradykinesia (slowness or poverty of movement), rigidity (resistance to passive movement), and akinesia (inability to initiate movement) which leads to postural and gait disorders.

The earliest evidence of a neurotransmitter mechanism in Parkinson's disease involved the clinical observation that reserpine could produce a Parkinsonism like syndrome as a dose dependent side effect (Hornykiewicz., 1973a). Reserpine had the unique capacity to deplete stores of catecholamines in the brain. Early lumbar CSF studies revealed decreased concentrations of the dopamine breakdown product Homovanillic acid (HVA) in lumbar cerebrospinal fluid (CSF) of PD patients (Davidson et al., 1977; Rinne et al., 1973; Pullar et al., 1970). Pathological studies revealed a 70-90% loss of dopamine cells in the mesencephalic brainstem (Nutt and Fellman., 1984). An 80-

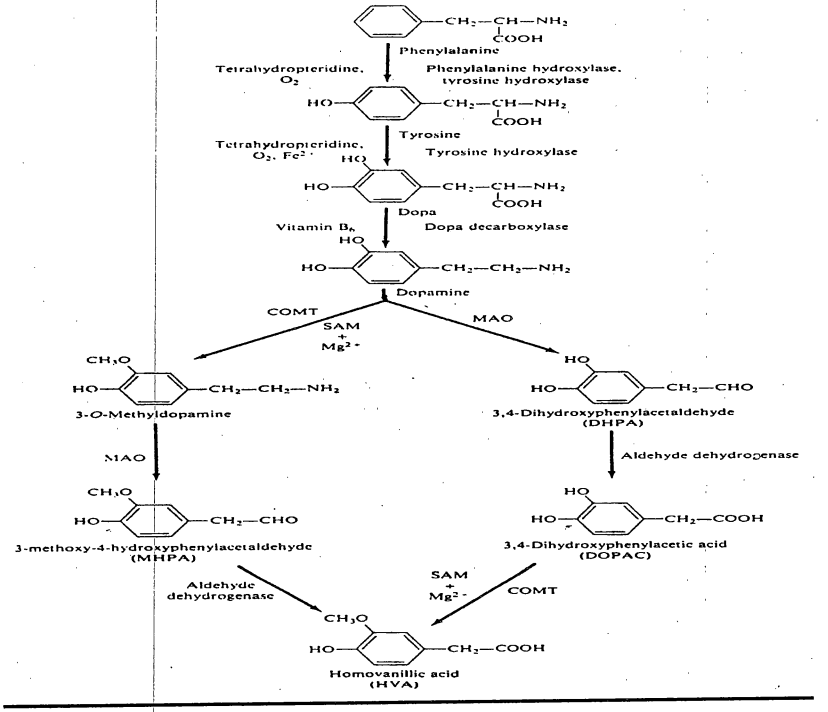
95% loss of dopamine in the striatum is thought to have occurred in Parkinsonian patients prior to the onset of symptoms (Hornykiewicz., 1973b). This evidence led to the discovery of the therapeutic efficacy of L-dopa, the immediate precursor to dopamine (see Figure 1), in the alleviation of the bradykinesia and rigidity associated with Parkinson's disease. In the intact striatum, the relative fraction of dopa-decarboxylase localized in interneurons may be far less than that in dopaminergic nerve endings (probably 10-20% of the total striatal decarboxylase activity, Melamed et al., 1980). This situation may be reversed after degeneration of the nigrostriatal system, and in Parkinsonism, interneurons may contain the major part of residual striatal decarboxylase. Since the dopamine molecules synthesized from L-dopa in these neurons probably cannot be stored in vesicular form, they would leak out, diffuse over short distances, become accessible to and stimulate dopaminergic receptors in the striatum, and thus mediate the therapeutic effects of systemically administered L-dopa (Melamed et al., 1980).

Symptoms of dyskinesia (involuntary choreiform /dystonic movements), on-off fluctuations, and hallucinations also commonly occur in Parkinsonian patients

on L-dopa therapy and are referred to as the L-dopa syndrome. Marked therapeutic efficacy is lost in greater than 50% of the patients three to five years after initial treatment with L-dopa (Fahn., 1981) and another 80% experience some of the severe motor and cognitive side effects (in addition to gastrointestinal and autonomic side effects) associated with L-dopa therapy within a year of the initiation of treatment (Cederbaum and McDowell., 1986). This has necessitated the characterization of the L-dopa syndrome, which manifests with symptoms such as severe dyskinesia, alterations in mentation, increased diurnal fluctuations, "on-off" periods, episodes of akinetic freezing and "crisis", increased fatigue and neurasthenia (Cederbaum and Schleifer., 1990). These can become the dominant and most disabling symptoms experienced by the patients. Because the treatment is also the cause, the only recourse is a reduction of the L-dopa, which has already lost much of its previous efficacy and has had little effect in improving, if not exacerbating, the non-medicated state. If the effectiveness of L-dopa indeed decreases course of the disease, it is probably not because it becomes less active on the symptoms it earlier improved but because it is relatively ineffective against a certain

number of Parkinsonian signs that gradually emerge. A study of basal and treated PD motor scores as a function of the duration of the disease has shown that the efficacy of L-dopa remains stable, at least for the symptoms that result predominantly from lesions of the nigrostriatal dopaminergic system (rigidity and tremor) as shown during non-dyskinetic "on" state (Agid et al., 1989; Sourkes and Poirier., 1966).

Figure 1. Biochemical Pathways of Biogenic Catecholamine Synthesis.

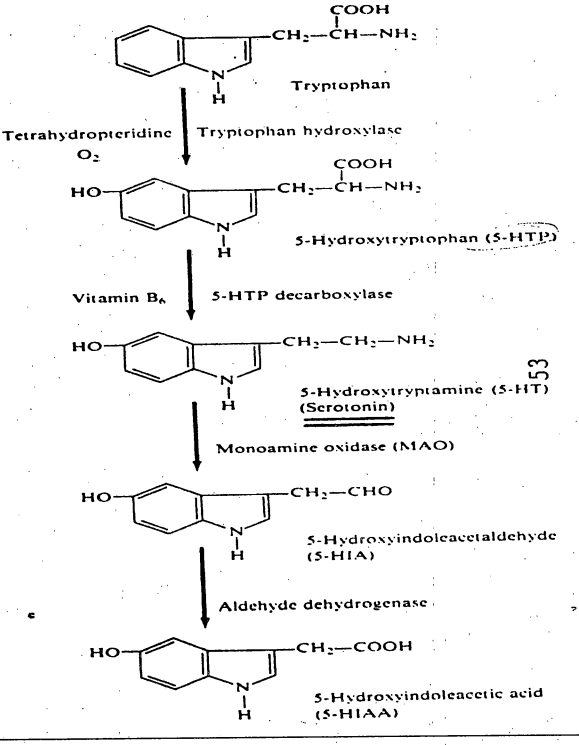


Note: Homovanillic acid (HVA) is a breakdown product of the dopamine. Enzymes are to the right of the arrows, co-factors are to the left.

Evidence for Serotonin (5-HT) Involvement in Parkinson's Disease

Although evidence for a serotonergic role in PD was found in the earliest studies, it received little attention until recently due to the early success of L-dopa therapy. The synthetic pathways for indoleamines is reviewed in figure 2.

Figure 2. Biochemical Pathway of the Synthesis of Biogenic Indolamines.



Enzymes involved in the reactions are on the right of the arrows, the co-factors are on the left. Major metabolite of the pathway is underlined.

L-Dopa Efficacy and Serotonin Metabolism Deficiency

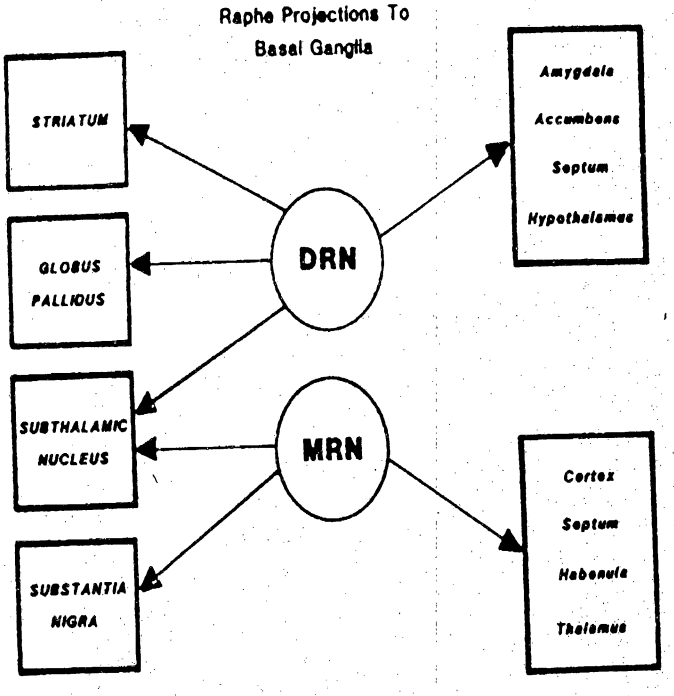
Several researchers have found that patients who become functionally independent on treatment with L-dopa had higher levels of the breakdown product of 5-HT, 5-hydroxyindole acetic acid (5-HIAA) than patients who showed no such improvement (Davidson et al., 1977), and suggested that intact 5HT neurons may be important in the therapeutic response to L-dopa (Davidson et al., 1977; Gumpert et al., 1973; Dray., 1981).

Pathological Studies

In PD, pathological lesions of dopamine containing cells in the substantia nigra and a loss of dopamine from the basal ganglia correlate with significant losses of serotonin and its major metabolite 5-HIAA from several brain regions including the striatum (40-90% 5-HIAA loss), globus pallidus, thalamus and substantia nigra (Fahn et al., 1971; Jellinger., 1968; DeLong and Georgopoulos., 1982). More recently researchers have found postmortem pathological evidence of a significant loss of serotonergic neurons (>50%) in discrete nuclei in the dorsal raphe nucleus (DRN), pontine nucleus oralis (PnO) and median raphe (MRN) in Parkinsonian patients (Wessemen et al.,

1990; Halliday et al., 1990b). The widespread innervation of the raphe nuclei is reviewed in Figure 3. Halliday et al., (1990a) concluded the same loss by correlating the neurotransmitter deficiency with the loss of serotonergic neurons (Halliday et al., 1990a).

Figure 3. Diagrammatic Representation of the Neural Connections of the Raphe Nuclei and the Basal Ganglia.



Dorsal raphe nucleus (DRN), Median raphe nucleus (MRN). Projections that arise from the DRN or MRN Are mainly unilateral and innervate many other areas of the forebrain as indicated.

Precursor Administration

The clinical precursor of serotonin, 5-hydroxytryptophan, when administered alone, does not appear to significantly alleviate the clinical symptoms of Parkinson's disease (Chase et al., 1972), though there are reports of exacerbation of tremor and/or rigidity (Chase.,1972) as well as reports of antidepressant effects, alleviation of tremor, no change and/or improvement in bradykinesia (Sandyk et al., 1986). It has been shown that the administration of 5-HTP to PD patients tends to reduces dopamine levels (McGreer et al, 1963, Sourkes et al, 1961). Similarly administration of levodopa causes a reciprocal reduction in serotonin metabolism (Wessemen et al., 1990). Thus it is likely that the administration of 5-HTP tends to reduce the levels of dopamine in the brain, in part due to the competition with catecholamine precursors for uptake into the central neurons as well as for decarboxylation to the amine (Chase et al., 1972). Investigations into the effects of nonspecific increases serotonin in the synapse, as would occur with 5-HTP therapy, revealed that the intensity of the negative feedback system of the autoreceptors precluded the absolute increase in synaptic serotonin (Aghajanian., 1978).

Selective Serotonin Reuptake Inhibitors (SSRIs)

Serotonin reuptake inhibitors are well recognized for their efficacy in treating depression (Lemberger et al., 1985; Lloyd et al., 1974), enhancing memory processing in mice (Flood., 1987), and have been reported to be effective in treating cataplexy, a sudden brief paralysis of voluntary muscle movement and loss of muscle tone (Langdon et al., 1986). Recent studies have shown that the hypokinetic symptoms of akinesia and bradykinesia, manifest as gait disorders, including gait freezing, postural instability, and masked facies experience significant alleviation following the administration of selective serotonin reuptake inhibitors (Iacono et al., 1994).

Subtypes of Parkinson's Disease

Within the broad spectrum of symptoms and manifestations of Parkinson's disease, two subtypes of Parkinson's disease are generally recognized and categorically designated Iacono type A and B. Those patients who present with a dominance of hyperkinetic motor symptoms (type A), such as tremor, dyskinesia, and cogwheel rigidity, typically present with a different prognosis, response to medication, and duration of disease before

incapacitation (see Table 1), distinguishable from those with the predominately hypokinetic motor symptoms (type B) such as bradykinesia, lead pipe rigidity, akinesia and postural instability (Iacono et al., 1995).

Table 1. Parkinson's Disease Subtypes.

Type A:	
Dominant symptoms:	tremor, cogwheel rigidity, some bradykinesia and Akinesia.
History:	typically greater than ten years since the onset of the disease with slow progression of tremor symptoms compared to other subtypes, with patient remaining ambulatory.
Medication response:	significant relief of\ hypokinetic symptoms with Sinemet.

Type A: (juvenile).	
Dominant symptoms:	include dyskinesia.
History:	onset before the age of forty. Presently the definition of juveniles stands at before 20, with less than forty, but greater than twenty at onset being "young onset".
Medication response:	on/off fluctuations with Sinemet.

Type AB:
Dominate symptoms: a combination of types A and B. Usually gait freezing is predominant with varying degrees of tremor, rigidity, and bradykinesia between patients.
History: Combination of A & B.
Medication response: Sinemet provides varying degrees of relief between patients.

Type B:
Dominant symptoms: rigidity, bradykinesia (slowness) and akinesia (characterized by Difficulty rising from a chair or turning in bed) are often particularly prominent while tremor is conspicuously absent or only mild in amplitude and frequency.
History: incapacitating symptoms develop less than ten years after onset with patients first experience being a loss of arm swing, or the dragging of a leg.
Medication response: Sinemet provides some alleviation of the symptoms, typically without the wide on/off fluctuations or prominent dyskinesia.

Parkinson's plus:	
Dominant symptoms:	are postural instability, autonomic symptoms and severe Incapacitation. MRI can be used to confirm the diagnosis by the presence of atrophy in the brain stem and/or striatum.
History:	with short history of PD symptoms as compared to other subtypes.
Medication response:	little or no relief with Sinemet.

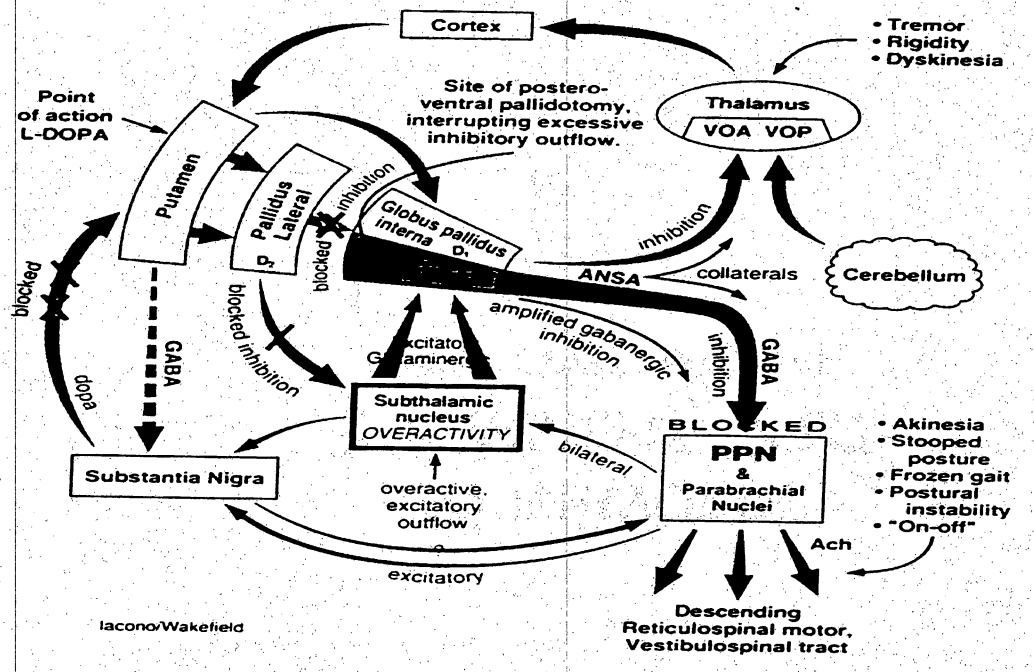
Note: Parkinson's disease subtypes as defined clinically by Dominant symptoms, history of disease progression and age of onset, and response to medication.

Type B patients typically manifest a dominance of axial symptoms and have diminished "hyperactivity" in the posteroventral pallidum. These patients include Jankovic's Postural Instability Gait Disorder Akinesia (PIGDA) (Jankovic et al., 1990). When the therapeutic response of type B patients was compared with that of type A, type B patients, not surprisingly, appeared to derive the most benefit from selective serotonin reuptake inhibitors. Interestingly, many of the type A patients reported an intolerance for SSRIs and an exacerbation of their tremor (Iacono, personal communication).

Parkinson's Disease
Hypothetical Model

The identified neurotransmitters involved in PD were elucidated by three signs; the presence of the neurotransmitter, high affinity uptake mechanisms of that neurotransmitter on the postsynaptic membranes, and the presence of the appropriate decarboxylase to degrade the neurotransmitter (Fonnum et al., 1978). The connections between the basal ganglia themselves were elucidated by autoradiographic and retrograde labeling techniques (Carter & Fibiger., 1978). The positive or negative influence of the neurotransmitter on the target neuron was deduced from changes in the target neuron degradative enzyme concentrations and receptor number regulation for the source neurotransmitter type due to kainic acid induced lesions (DiChiara et al., 1980; McGeer et al., 1978), and surgical lesions in various mammals (Penney & Young., 1983). The pathways were shown diagrammatically in Figure 4.

Figure 4. Current Model of Neurochemical Pathways in Parkinson's Patients.



Metabolites: Levo Dopa (L-dopa), Gamma aminobutyric acid (GABA), Acetylcholine (Ach). Tracts: Ansa lenticularis (ANSA). Nuclei: Pedunculo-pontine nucleus (PPN), Ventriculo-opthalmic Anterior nucleus (VOA), Ventriculo-opthalmic posterior nucleus (VOP). Arrows indicate direction of the flow of neurotransmission. (From Iacono et al., *Neurosurgery*, Vol 36, No. 6, 1995) reproduced with permission.

A loss of dopamine leads to the overactivity of the striatal inhibitory projections to the external globus pallidus by two mechanisms: First the excitatory acetylcholinergic interneurons in the striatum would no longer be negatively modulated and would increase inhibitory GABAergic striatopallidal efferent activity.

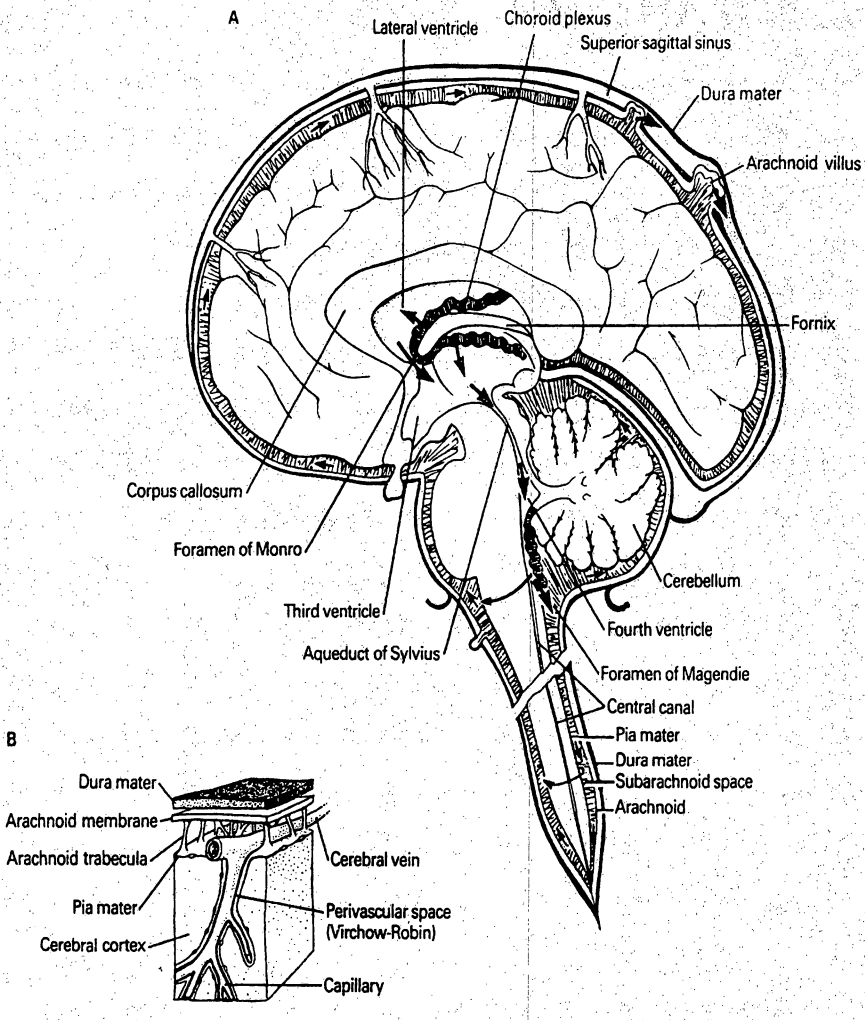
Secondly, the loss of nigrostriatal dopaminergic afferents or dopamine released in the striatum would disinhibit GABAergic striatopallidal efferents (Penney & Young., 1983). This results in increased inhibitory outflow of the unmodulated striatopallidal projection to the external pallidum, via the indirect pathway. The increased inhibition of the external pallidum, subsequently, results in decreased GABAergic output, which disinhibits (activates) the subthalamic nucleus to which it projects. The primarily glutaminergic fibers of the subthalamic nucleus monosynaptically project to, and amplify, the neuronal activity of the internal globus pallidus. Single unit microelectrode recording from the subthalamus and medial globus pallidus has revealed excessive neuronal firing in both structures relative to their intact controls (primate MPTP model) (Miller & DeLong., 1987). Ultimately, excessive GABAergic inhibition of the pedunculopontine nucleus is achieved via enhanced ventral pallidal outflow through the ansa lenticularis (Iacono et al., 1995). The findings of Pasik et al., (1984) showed the location of 5HT immunoreactive terminals in the medial globus pallidus. When taken together with the biochemical results of predominant lowering of pallidal 5HT levels in

PD (Fahn et al., 1971) they emphasize the importance of the serotonergic innervation of the globus pallidus for the normal functioning of this model.

Effects of PosteroVentral Pallidotomy (PVP)

PVP potentially reduces the pathologically amplified GABAergic outflow from the internal pallidum, allowing disinhibition of the PPN (locomotor regulating region) (see Figure 4). Concomitant interruption of collaterals to ventral lateral thalamus accounts for alleviation of the hyperkinetic symptoms (which predominate in Iacono type A and are also alleviated with thalamotomy) such as tremor, rigidity and dyskinesia as well as the reversal of akinesia (Iacono et al., 1995).

Figure 5. Neuroanatomy of CSF Production and Ventricular System of the Human Brain.



Note: position of the third ventricle where vCSF samples were taken from in the Parkinson's type A and B patients undergoing Postero ventricular pallidotomy. Arrows indicate direction of CSF flow. Reproduced with written permission from Kandel, E.R, Schwartz, J.H., Jessell, T.M. In *Principles of neural Science*. Third edition. Appleton & Lange. Pg 1051.

CSF Analysis

Many researchers have investigated the concentrations of various biogenic amines in cerebrospinal fluid (see Figure 5) of PD sufferers hoping to elucidate a quantifiable neurochemical imbalance that might shed light on the mechanism of the disorder. CSF Levels of both 5-HIAA and serotonin depend upon the degree of activity in the central nervous system (CNS), therefore a positive correlation of serotonin and 5-HIAA concentrations to level of activity would be expected (Volicer et al., 1985a).

Particular attention has been paid to the levels of 5-HIAA in PD patient CSF, since it is the final breakdown product of the serotonin pathway. However there have been conflicting reports as to whether lumbar CSF 5-HIAA concentration is lower in PD sufferers than compared to controls (Gottfries et al., 1969; McCantz et al., 1992, Kostic et al., 1987) or not significantly different (Ogawa et al., 1992; Toghi et al., 1993). Other discrepancies have occurred in regards to correlation with symptoms, some researchers have reported that low 5-HIAA levels are closely linked to mood and not movement disorders (Ashcroft et al., 1966). Mayeux et al., (1986) investigated the lumbar CSF concentrations of normal, PD patients and PD

patients with depression. They found that the 5-HIAA content of CSF is 50% lower in non-depressed PD patients compared with normal controls. Patients with PD and major depression had CSF 5-HIAA levels 70% lower than the normal controls (Mayeux et al., 1986), suggesting that PD related depression appears late in PD symptom progression. More recently it has been reported that serotonin is more closely correlated to gait freezing than dopamine (Toghi et al., 1993). More recent studies have shown that the concentration of 5-HTP, total serotonin, kynurenine, 3OH kynurenine (but not 5-HIAA) seem to be significantly reduced in Parkinson's patient lumbar CSF as compared to normal patients (Ogawa et al., 1992; Toghi et al., 1993).

The current model for PD suggests that serotonin plays a significant role in type B Parkinson's disease, but not in type A. If this is so we would expect to find significantly lower levels of 5-HIAA and serotonin in the ventricular CSF (vCSF) of type B as compared to type A PD patients (Our hypothesis). We investigated the ventricular concentration of 3-OHK, 5-HTP and HVA to allow us understand the biochemical interactions of these metabolites in the serotonin biosynthetic pathway and how they relate to Parkinsonism.

CHAPTER TWO

MATERIALS AND METHODS

Patient Base

We studied 23 patients taking Sinemet (Carbidopa + L-dopa) with an average age of 66.8 years. The average duration of the disease was 10.8 years. None of the patients in the study were on serotonin reuptake inhibitors or tricyclic antidepressants. We used the Unified Parkinson's Disease Rating Scale (UPDRS) for evaluating individual symptoms.

Sample Collection

CSF samples were obtained from the third ventricle using a catheter inserted prior to the injection of dye for ventriculography, a localizing technique in Posteroventral Pallidotomy (Iacono et al., 1995). Samples were collected with a sterile syringe by the neurosurgeon. The first 200ul or so was discarded to avoid contamination of the CSF sample with blood cells from the insertion of the catheter. We then connected a sterile 5ml syringe to the catheter and withdrew 3-4ml of CSF. We then transferred the CSF to three microcentrifuge tubes, which were then centrifuged at

16000g for 8 minutes to remove remaining blood cells, ependymal wall cells and platelets. The supernatant (CSF) was then dispensed in 150ul portions into microcentrifuge tubes already containing 150ul of our mobile phase (pH 3.4), mixed and immediately snap frozen with liquid nitrogen in the operating room. The samples were then moved to a -70 degree Celsius freezer until analysis. The acidity of the mobile phase and the extremely low temperatures (and the darkness inside the freezer) essentially eliminate spontaneous degradation of the CSF collected (Jakubovic et al., 1987).

Preparation of Stock Solutions, Standards and Mobile Phase

All solutions were made to the specifications outlined in Table 2 below.

Table 2. Solutions Used in this Study.

Stock solutions: 0.8M KH_2PO_4 Stock soln. (2L) 0.15M EDTA Stock soln. (50ml) 0.2M OSA Stock soln. (50ml)
Mobile phase soln. (3Liters) pH 3.5, 9.5% methanol, 0.2uM EDTA, 0.15uM OSA, 0.1M KH_2PO_4

Standard solutions: 50ml solutions (8ng/ml), refrigerated till use:
- 5-Hydroxytyramine
- 5-Hydroxyindol-3-aceticacid
- 5-Hydroxy-L-tryptophan
- 3-Hydroxykyneurenine
- Homovanillic Acid
- N-omega-methylserotonin

Sample Spiking

I thawed the samples (50:50 CSF:MP), then vortexed them to ensure they were thoroughly mixed. I then transferred 150ul of the sample into Millipore microcentrifuge filter and tube apparatus. To this we added 150ul of N-omegamethyl serotonin (NOM), (concentration 800pg/100ul) which will be my internal standard and a control for loss of metabolites. I then centrifuged the filtration apparatus at 16000 g. for 2 minutes, then discarded the filter. The filtrate was then used for injection purposes to determine concentration of metabolites in the CSF.

Data Collection

Reverse phase high performance liquid chromatography coupled with electrochemical detection has been used successfully to measure the concentrations of various

biogenic amines and their metabolites in various human and animal biological samples (Jakubovic et al., 1987).

HPLC techniques are either gradient or isocratic with respect to the mobile phase pH employed. For our investigations we have developed and utilized an isocratic system capable of resolving several biogenic amines and their metabolites including; 3OHK(2.7), 5HTP(5.0), 5-HT (10.0), 5-HIAA(11.0), Nomegamethyl serotonin (12.0), HVA(19.0). The numbers in brackets represent approximate retention times in minutes.

Concentrations of 3-OHK, 5-HTP, 5-HT and 5-HIAA were determined by injection of 50ul volumes of the 25% CSF solution into a reverse phase C18 Novapak column (Novapak, Waters). Sample analysis was performed using isocratic high performance liquid chromatography (HPLC) with a glassy carbon electrochemical detector. The mobile phase consisted of 0.1M potassium phosphate (monobasic), 0.15uM orthosulfonic acid (OSA), 0.2uM ethylenediaminetetraacetic acid (EDTA) and 9.5% ethanol at pH 3.4. The working electrode was set to a potential of 0.70mV. The column and the electrodes were at room temperature. All standards were obtained from Sigma Chemical Company. The detection limit was 20pg/ml and the recovery rate was almost

100%. The HPLC-EC equipment was set to the configurations outlined in table 3.

Table 3. HPLC-EC Analytical System Configuration.

HPLC system.	
Pump:	Waters 510
Detector :	Waters 460 Electrochemical detector
Column:	Novapak
Guard Column:	uBondpak
Autosampler:	Waters 700 WISP Satellite

Detector conditions	
Reference potential:	0.70mV
Working potential:	0.704mV
Sensitivity:	10nA full scale deflection
Filter setting:	1
Time Constant :	0.5

Chromatography:	
Mobile Phase:	pH 3.5, 9.5% methanol, 0.2uM EDTA, 0.15uM OSA, 0.1M KH2PO4
Flow Rate:	1.0mL/min
Pressure:	1500 psi

Data Analysis

The data were analyzed using regression and one tailed t-test functions in Microsoft Excel.

Validation of Experimental Conditions

The validity of the data is dependant upon the quality of the experimental conditions. Several experiments were

conducted to eliminate potential contamination and degredation problems.

Blood Contamination of Samples

Occasionally it is not possible to get "clean" (blood cell free) samples of CSF from the catheter leading to the third ventricle. This usually results from several passes prior to successful catheterization of the ventricle. Blood platelets and RBCs have serotonin receptors which can bind serotonin and thus reduce the level of serotonin detected in blood contaminated CSF. Similarly blood platelets contain high levels of 5-HIAA and could throw off the data should they lyse. Normally blood plasma and CSF are in osmotic equilibrium hence we can remove blood cells from the CSF by filtration without lysing the cells before stabilizing the CSF with acid (mobile phase) using microcentrifuge filtration of 1.5ml aliquots at 16000g for 8 minutes.

Optimum Sample Injection Volume

The samples were already stabilized in a 50% CSF to 50% mobile phase solution. We injected amounts ranging from 10 to 150 micro liters. We looked at the peak heights

to see if they were within the detectable range. We then looked carefully at the baseline separation and determined the volume that optimized resolution while still affording measurable concentrations of the metabolites under investigation.

Filter Contamination

We ran several straight injections of mobile phase directly from the auto sampler vials without passing through the micro centrifuge tubes and filters. We also ran mobile phase that had been passed through the 0.22um micro centrifuge filters. The resulting chromatograms clearly demonstrated that there was a contaminant from the filtration process being eluted at 17.1 minutes. We elected to continue to use the filters since the elution time of the contaminant clearly did not interfere with any of the retention times of our standards.

Degradation and Filter Contamination Elution

We performed several tests to see if there was any degradation of the samples while awaiting injection into the auto sampler. We ran a test set of injections: the mixed standard (50ul injection), then a 3hydroykynurenine

(3OHK) run (50ul injection) , followed by five spiked samples in a row (100ul injection). This was followed by an immediate replicate set of injections and a final mixed standard injection and 3OHK Injection. We attempted to elucidate whether contaminants obtained from the filter could be eluted out through successive filtering. We thawed a sample, vortexed it and the centrifuge filtered it into a micro centrifuge tube. We then filtered an identical quantity of the same CSF through the same filter into another micro centrifuge tube. This procedure was repeated five times at which point the filter became clogged with minute tissue particles. We found that the contaminant peak at seventeen minutes did not appreciably change in size. This would indicate that there is no decrease in the contamination that results on re-use of the filters, or more likely that it is the micro centrifuge tubes themselves that have something on them that causes the peak at seventeen minutes and that it is a very consistent amount of contamination.

3-Hydroxykyneurenine Degradation Analysis

We ran the same 3OHK sample three times over a 500-minute (6.3 hrs) period. During this time the samples were

in amber injection vials, in the auto sampler carousel at room temperature. The first 3OHK run was after 125 minutes in the auto sampler. The second was at 300 minutes, and third run was at 475 minutes. Upon analyzing the peak heights of the 3OHK standard we found that 3OHK was completely gone within the eight hour period it took the auto sampler to inject the final 3OHK sample, and more than three quarters gone after 5 hours. The test was to elucidate the degradation that may occur during extended times exposed to room temperature while awaiting analysis. The amber vial nullified photodegradation (Jakubovic et al., 1978).

Mixed Standard Degradation

We ran mixed standard injections from amber vials in an auto sampler at time intervals of 100, 275, and 450 minutes. The degradation of 3OHK was most profound and mirrored that of the solo 3OHK standard degradation experiment. The 5HTP serotonin and HVA peaks appeared to be stable over the eight-hour period prior to injection. 5-HIAA concentration in the mixed standard appeared to be slightly decreased after eight hours. We did not go beyond 500 minutes at any time since no sample would be kept

unrefridgerated awaiting analysis in the actual data collection phase of this study. Our results lead us to keeping times in the auto sampler down to less than 250 minutes.

Sample Vial Standardization

To test for potential insert contamination we ran ten different inserts containing mobile phase. Four inserts had been washed with mobile phase prior to injection. Three inserts were straight from the factory, and three inserts were acid washed, rinsed and dried before filling with mobile phase. Our results showed that though some vials had slight contamination, all contaminants were eluted within the first two minutes. We also found that the cleanest vials were those that had been acid washed then dried. We resolved to acid wash the vials using the following protocol prior to use:

Vials were placed in a glass-stoppered jar containing 6N HCL. After 2 days the vials were transferred to a glass stoppered jar containing distilled deionized water. The vials were allowed to soak in the water for one week and were then rinsed with distilled deionized water and placed in a clean plastic box to dry prior to use.

Linearity and Sensitivity

We prepared dilutions of the stock solutions to allow for sensitivity studies. Conditions and results are shown in Table 4.

Table 4. Linearity Study Solution Concentrations

Vial	stk	vol	MP	TVol	Ivol	MSpg	HVApg
1	SMX	150	150	300	50	600	1200
2	SMX	100	200	300	50	400	800
3	SMX	50	250	300	50	200	400
4	3	75	275	300	50	50	100
5	4	75	225	300	50	12.5	5
6	5	75	225	300	50	3.125	6.25
7	1	75	225	300	50	150	300
8	7	50	250	300	50	25	50
9	8	75	225	300	50	6.25	12.5
10	9	75	225	300	100	3.125	3.125

Stock solution SMX contained all standards at a concentration of 2400pg/100ul,except HVA, which was 4800pg/100ul.

CHAPTER THREE

RESULTS

Linearity

The table shows that the area under the peaks obtained for the standard solutions correlated linearly with the concentrations of the standard solutions employed.

Table 5. Linearity of Peak Integration to Standard Solution Concentration.

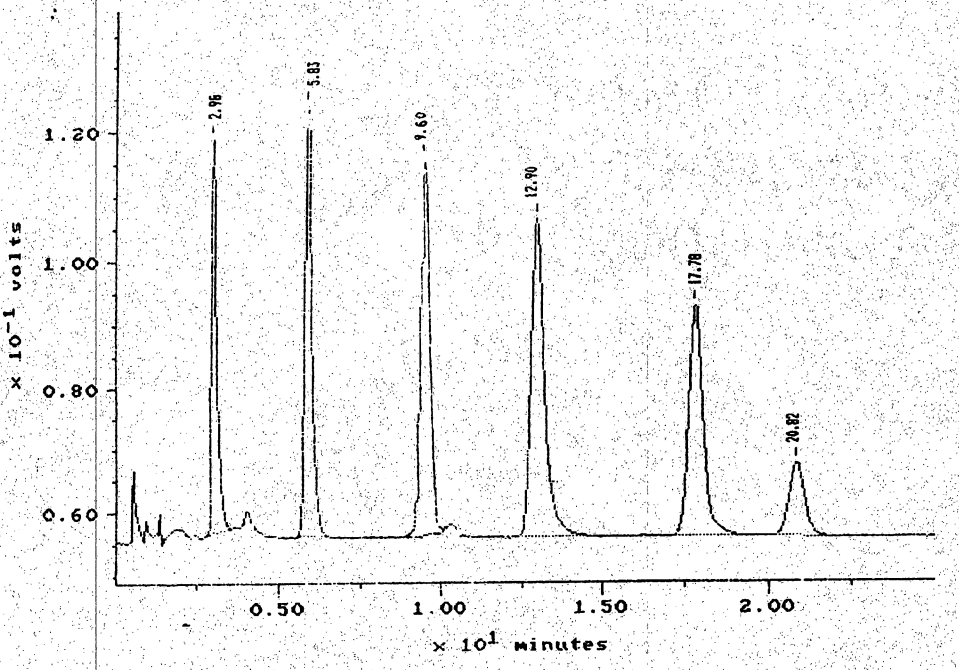
Linearity

Metabolit	R Square
HVA	0.998719
NOM	0.996988
5HTP	0.997876
3OHK	0.998207
5HIAA	0.998417
SER	0.997558

Metabolites: Homovanillic acid (HVA),
N-omega-methylserotonin (NOM),
5-hydroxytryptophan (5HTP),
3-hydroxykyneurenine(3OHK),
5-hydroxyindoleacetic acid (5HIAA),
Serotonin (SER).

The following chromatogram shows the HPLC-EC output for the mixed standards used to calculate linearity. The retention times in minutes for the metabolites were as follows: 3OHK (2.96), 5HTP (5.83), 5-HT (9.60), 5-HIAA (11.0), NOM (17.78), HVA (20.82).

Figure 6. Sample Chromatogram of the Mixed Standards for Linearity.



Retention times: 3OHK (2.96), 5HTP (5.83), 5-HT (9.60), 5-HIAA (11.0), NOM (17.78), HVA (20.82).

Statistical Analysis of vCSF Metabolites in Type A PD Patients

Type A patient vCSF levels of 3-OHK were 7.11 ng/ml (Table 6). The range of values was 0.7 to 24.9 ng/ml and the standard error was 1.8.

The average concentration of 5-HTP in type A patient vCSF was found to be 14.1ng/ml (Table 6). Our standard

deviation was 4.3ng/ml, and the range of values was between 1.3 to 61.7ng/ml.

The concentrations of serotonin in the VCSF of type A patients averaged 1.65ng/ml (Table 6).

The values we obtained for the concentration of 5-HIAA in type A patients was 104.4ng/ml (Table 6). The range of values was 55 to 157ng/ml, and the standard error was 10.

The concentration of NOM in type A vCSF averaged 18ng/ml, the range was 15.4 to 21ng/ml, and the standard error was 0.6. Indicating that out sample spiking was consistent.

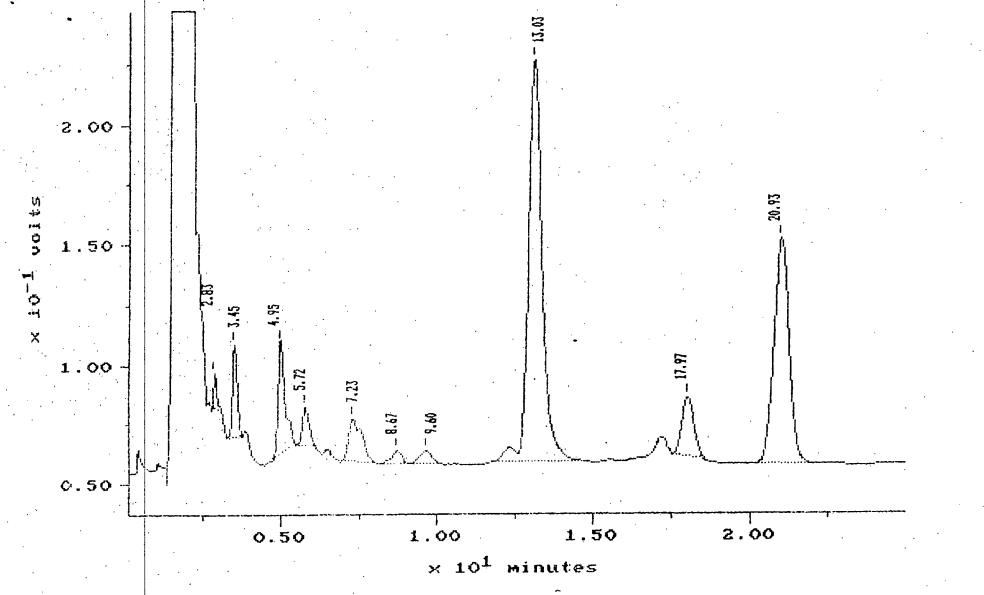
The average vCSF HVA concentrations of type A patient values were 1007.5ng/ml (Table 6). Range of values was 416 to 2333ng/ml. The standard error was 176.

Table 6. Integration for HPLC Output in ng/ml, PD Type A.

Parkinson's Disease type:	A					
	3OHK	5HTP	5-HT	5HIAA	NOM	HVA
4	24.936	12.304	0.664	91.4	15.432	762.872
8	6.152	6.216	1.976	55.264	16.912	624.96
11	8.416	10.6	0.112	168.96	21.224	1536.912
12	3.304	12.112	0.264	65.624	17.632	416.752
14	3.288	14.488	3.008	143.512	18.016	1818.048
15	6.56	18.96	0.52	143.032	19.544	1776.464
19	15.872	2.304	1.32	75.248	19.016	428.392
20	2.72	1.336	2.688	97.352	12.928	765.424
25	3.416	11.584	5.08	157.456	19.968	2333.144
26	5.392	3.208	1.728	87.928	19.392	426.68
27	0.696	21.808	0	106.656	20.248	971.096
32	8.72	7.536	0.544	81.072	19.92	628.152
36	3.04	61.76	3.52	79.76	18.32	608.4
Average	7.116308	14.17046	1.648	104.0972	18.35015	1007.484
Standard	1.830516	4.316261	0.430309	10.25886	0.622797	176.8111

Parkinson's disease type A patients (a), Parkinson's disease type B patients (b),
3- hydroxykyneurenine(3OHK), 5-hydroxytryptophan (5HTP), Serotonin (5-HT), 5-hydroxyindoleacetic acid (5HIAA), N-omega-methylserotonin(NOM), Homovanillic acid (HVA),

Figure 7. Sample Chromatogram of PD Type A Patient vCSF.



Retention times: 3OHK (2.8), 5-HTP (5.7), 5-HT (9.6), 5-HIAA (13.0), HVA (20.9).

Statistical Analysis of vCSF Metabolites in Type B PD Patients

Type B patient vCSF levels of 3-OHK were 7.81 ng/ml, on average, the range was 0.8-14 ng/ml, and the standard error was 1.23ng/ml.

The average concentration of 5-HTP in type B patients was 524ng/ml, the range was 1.6 to 3083ng/ml (Table 7). The standard error was slightly higher than the mean at 266ng/ml.

The concentrations of serotonin in the vCSF of type B patients was 0.83ng/ml (Table 7). The average concentration of 5-HT in type B patient vCSF was 0.25 to 1.6ng/ml, and the standard error was 1.7ng/ml.

The values we obtained for the concentration of 5-HIAA in type B patients was 105.7ng/ml (Table 7). The range was 65 to 195ng/ml, and the standard error was 9.5ng/ml.

Type B NOM spike concentration averaged 19.7ng/ml, the range was 12-23ng/ml and the standard error was 0.8ng/ml (Table 7). This indicates that the spiking was reasonably consistent.

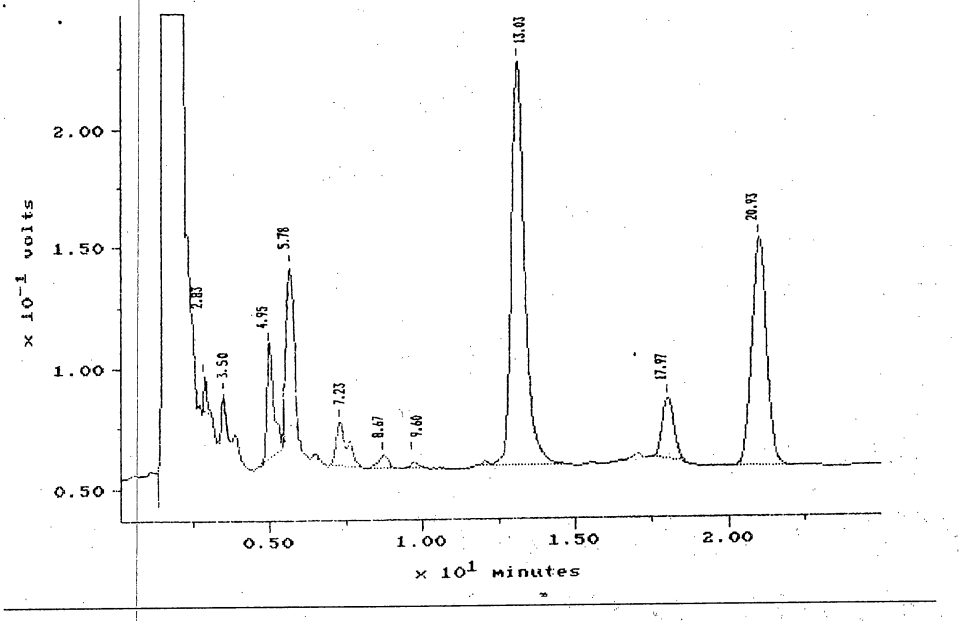
The vCSF HVA concentrations of type B patient average values we obtained was 882ng/ml. The range of values was 330-1522ng/ml and the standard error was 143ng/ml.

Table 7. Integration for HPLC Output in ng/ml, PD Type B.

Parkinson's Disease type: B						
Patient #	3OHK	5HTP	5-HT	5HIAA	NOM	HVA
1	4.28	349.151	0.25232	122.752	18.496	641.264
2	12.728	10.40256	0	65.752	16.024	635.44
5	7.992	303.1258	0.85656	75.136	20.024	581.512
10	9.592	1.57248	2.0252	102.36	21.624	1124.944
18	11.848	36.10656	0.51128	121.568	20.864	716.568
21	6.52	5.63808	0.71712	121.912	21.968	330.024
22	12.872	1979.988	1.16864	118.464	21.256	1007.112
23	0.84	3083.459	0.39176	113.68	23.056	502.792
24	6.952	1.23648	0.54448	94.408	19.472	780.208
29	9.192	9.78432	1.06904	78.896	20.112	671.944
34	14.16	21.00672	1.38112	195.984	23.04	2273.76
35	2.6	9.18624	0.24568	66.816	18.384	684.832
41	1.96	1014.162	1.63344	96.416	12.136	1522.912
Average	7.810462	524.986	0.830511	105.7034	19.72738	882.5625
Standard	1.23092	266.6208	0.166067	9.530138	0.836911	143.2381

Parkinson's disease type A patients (a), Parkinson's disease type B patients (b), 3-hydroxykyneurenine(3OHK), 5-hydroxytryptophan (5HTP), Serotonin (5-HT)., 5-hydroxyindoleacetic acid (5HIAA), N-omega-methylserotonin(NOM), Homovanillic acid (HVA),

Figure 8. Sample Chromatogram of PD Type B Patient vCSF.

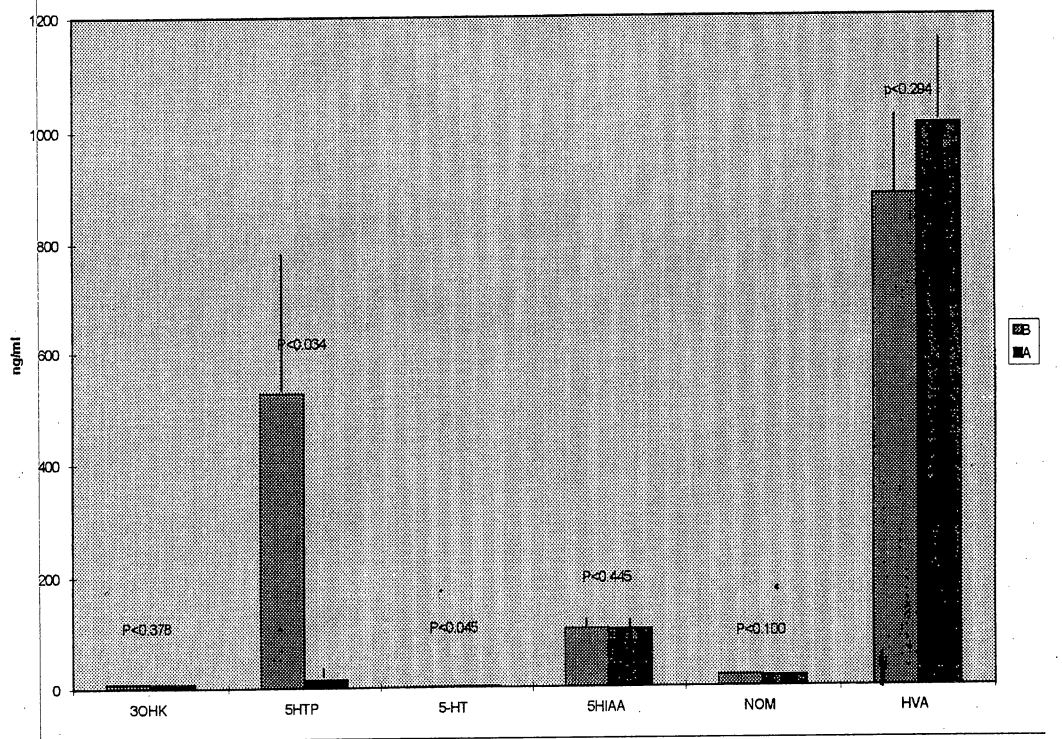


Retention times: 3OHK (2.8), 5-HTP (5.8), 5-HT (9.60), 5-HIAA (13.0), NOM (18.0), HVA, (20.9).

Comparison of Type A versus Type B Parkinson's Patient vCSF

We found a significant increase in the amount of 5-HTP ($p<0.034$) in ventricular CSF (vCSF) of PD type B patients as compared to type A patient vCSF (Figure 9).

Figure 9. Graphical Comparison of Type A versus Type B Parkinson's Patients vCSF.



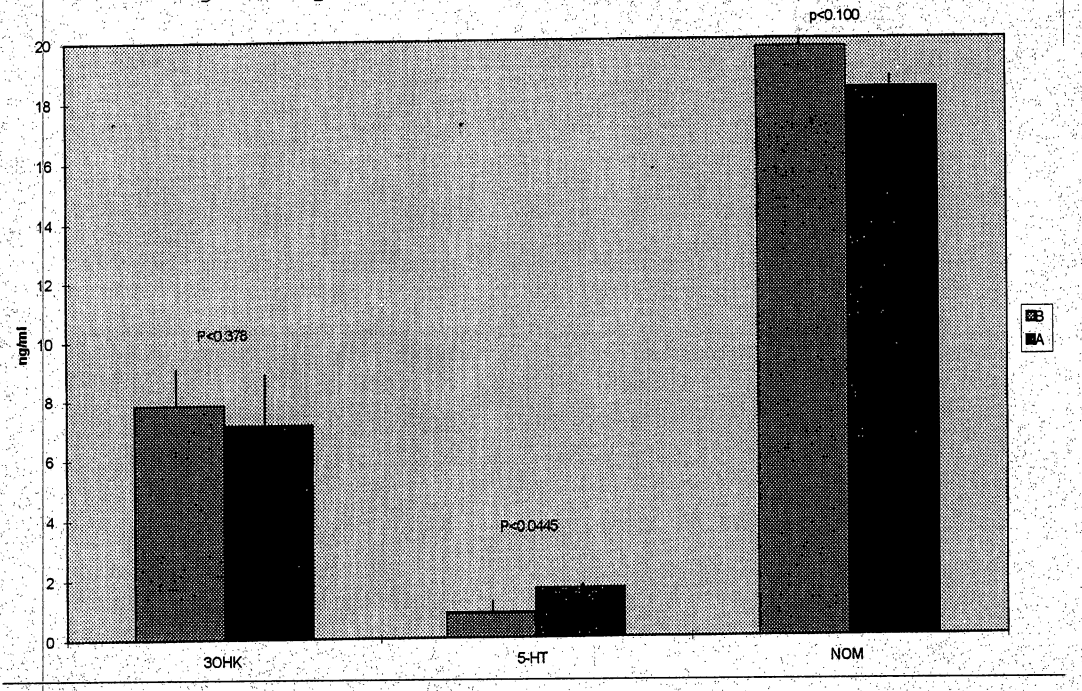
Parkinson's disease type A patients (A), Parkinson's disease type B patients (B), 3-hydroxykyneurenine (3OHK), 5-hydroxytryptophan (5HTP), Serotonin (5-HT), 5-hydroxyindoleacetic acid (5HIAA), N-omega-methylserotonin(NOM), Homovanillic acid (HVA),

Comparison of Type A versus Type B PD vCSF Concentrations of 3-Hydroxykyneurenin, Serotonin and N-Omegamethylserotonin

We did not find any statistically significant difference in the concentrations of 5-HIAA and HVA between type A and B Parkinson's disease patients. The concentrations of 3OHK, 5HT and NOM are graphically magnified in the following bar chart (Figure 7).

We found a significant reduction of 5-HT ($p<0.045$) in ventricular CSF (vCSF) of PD type B patients as compared to type A patient vCSF. We did not find any statistically significant difference in the concentrations of 3-OHK and NOM between type A and B Parkinson's Disease patient vCSF.

Figure 10. Graphical Comparison of Type A versus Type B Parkinson's Patients vCSF Concentrations of 3-Hydroxykyneurenine, Serotonin and N-Omegamethylserotonin.



Parkinson's disease type A patients (A), Parkinson's disease type B patients (B), 3-Hydroxykyneurenine (3OHK), Serotonin (5-HT), N-Omegamethylserotonin(NOM).

Summary of vCSF Metabolite Concentrations in Type A and B PD Patients

We found a significant increase in the amount of 5-HTP ($p<0.034$) and reduction of 5-HT ($p<0.045$) in ventricular CSF (vCSF) of PD type B patients as compared to type A patient vCSF (Table 8). We did not find any statistically

significant difference in the concentrations of 5-HIAA, HVA and 3-OHK between type A and B Parkinsonism.

Type B levels of 5-HT were, on average, 50% lower than that of type A patients (0.83ng/ml and 1.65ng/ml respectively).

Table 8. Statistical Comparison of Third Ventricle CSF Metabolic Amine Concentrations Between Type A and B Parkinson's' Disease Patients.

Significance	
t-Test: Two-Sample Assuming Equal Variances	
CSF metabolites	**P(T<=t) one-tail**
3OHK (a) 3OHK (b)	0.377861
5HTP (a) 5HTP (b)	0.0337
5-HT (a) 5-HT (b)	0.044515
5HIAA (a) 5HIAA (b)	0.454816
NOM (a) NOM (b)	0.099614
HVA (a) HVA (b)	0.294044

Parkinson's disease type A patients (a), Parkinson's disease type B patients (b), 3-hydroxykyneurenine(3OHK), 5-hydroxytryptophan (5HTP), Serotonin (5-HT), 5-hydroxyindoleacetic acid (5HIAA), N-omega-methylserotonin(NOM), Homovanillic acid (HVA).

PD vCSF Metabolite Concentrations Compared to Literature Values for Normal Adults

The average concentration of 5-HTP in type A patient vCSF was found to be 14.2ng/ml, which falls in the normal

range of 1.1 to 68.9 nanomoles per liter, (0.24 to 15.2ng/ml) described by Koskiniemi et al., (1985). In contrast our type B patient vCSF average 5-HTP concentration was found to be some 34 times higher than normal at 525ng/ml.

The average concentration of 5-HT in type B patient vCSF was close to the bottom 1/6th of the normal range (0.34-4.8ng/ml) reported by Koskiniemi et al., (1985), whereas the average type A concentration was above the bottom 1/3rd.

The values we obtained for the concentration of 5-HIAA in both type A and type B patients (104.4 and 105.7ng/ml respectively) were at the high end of the range for normal vCSF reported by Koskiniemi et al., (85.2-105.2ng/ml), and in the top third of values reported by Bouckoms et al., (60-120ng/ml) (Bouckoms et al., 1993).
The average values we obtained, (1007.5ng/ml and 882.6ng/ml type A and B respectively), were outside of the normal range of 150-550ng/ml reported by Bouckoms et al., (1993).

There is no available data for vCSF 3-OHK levels in the literature. Toghi et al (1993) reported lumbar CSF levels of 3-OHK decreased significantly in the PD patients as opposed to their controls ($p<0.05$), with concentrations

of 0.24 +/- 0.16ng/ml and 1.37 +/- 0. 63ng/ml for PD patients and controls respectively. Our values for vCSF 3-OHK concentrations were 7.11 and 7.81 ng/ml for type A and type B patients respectively indicating that the concentration of 3-OHK in vCSF is much greater than in lumbar CSF.

Table 9. PD vCSF Metabolite Concentrations Compared to Literature Values for Normal Adults.

CSF Biogenic Amine Metabolite Concentrations (ng/ml)					
	3-OHK	5-HTP	5-HT	5-HIAA	HVA
Bouckoms et al.				60-120	150-550
Koskiniemi et al.		0.24-15.2	0.34-4.8	85.2-105.2	
Iacono PD type A	7.1	14.2	1.6	104.1	1007.5
Iacono PD type B	7.8	525	0.8	105.7	882.6

Parkinson's disease type A patients (a), Parkinson's disease type B patients (b), 3-hydroxykyneurenine (3OHK), 5-hydroxytryptophan (5HTP), Serotonin (5-HT), 5-hydroxyindoleacetic acid (5HIAA), Homovanillic acid (HVA).

Regression Analysis

Regression analysis did not reveal a correlation between any of the serotonin pathway metabolite concentration in vCSF (Table 10). We also found no

correlation between levels of 5-HTP and 3-OHK, 5-HTP and HVA, 5-HTP and number of years since disease onset.

Table 10. Regression Analysis by Correlation Between Metabolites.

Regression Statistics		
CSF metabolites		**R Square**
5HTP (b)	5-HT (b)	0.000114
5-HT (b)	5HIAA (b)	0.090507
5HTP (b)	5HIAA (b)	0.0095
5HTP (b)	HVA (b)	0.00537
5HTP (a)	5-HT (a)	0.053801
5-HT (a)	5HIAA (a)	0.030215
5HTP (a)	5HIAA (a)	7.3E-05
5HTP (b)	3OHK (b)	0.127649
HistoryB	5HTP (b)	0.038553

Parkinson's disease type A patients (a),
Parkinson's disease type B patients (b)
3-hydroxykyneurenine(3OHK), 5-hydroxytryptophan (5HTP), Serotonin (5-HT).,
5-hydroxyindoleacetic acid (5HIAA), Homovanillic acid (HVA).

CHAPTER FOUR

DISCUSSION

Ventricular Cerebrospinal Fluid (vCSF)

The CSF itself is secreted by the choroid plexus into four ventricles (see Figure 5). Extrachoroidal CSF is secreted by brain capillaries. CSF flows through the intraventricular foramina (of Monro) into the third ventricle and then into the fourth ventricle through the cerebral aqueduct (of Sylvius) through the foramina of Magendie and Lushcka into the subarachnoid space (see Figure 5). The subarachnoid space lies between the arachnoid and the pia mater which together with the dura mater form the three meninges that cover the brain. The CSF circulates to the subarachnoid space and is absorbed into the venous system via the arachnoid villi, which are situated adjacent to the spinal roots. Within the subarachnoid space the CSF flows down the spinal canal and also up over the convexity of the brain. CSF is formed at a rate of about 500ml/day and flows one way from the subarachnoid space into the venous capillaries (bulk flow), hence it is understood that metabolites formed in the lumbar CSF do not eventually flow up into the ventricular

CSF. It is then a question as to what degree the lumbar CSF reflects brain levels (vCSF). To answer this question Volicer et al., (1985b) used various fractions of CSF derived by lumbar puncture. They found that the concentrations of many of the metabolites varied with the proximity of the brain . For example the 20th milliliter was more representative of the ventricular CSF (Volicer et al., 1985b). This would indicate that the use of lumbar CSF samples to indicate brain concentrations of biogenic amine metabolites are not particularly useful (Bulat and Zivkovic., 1971). The presence of metabolites in vCSF is due to leakage from the surrounding brain tissue and hence is ideal for analysis. We decided not to investigate amino acid precursors in the vCSF since the uneven reduction in serotonin and dopamine throughout the neostriatum found in PD patients (Fahn et al., 1971) would appear to preclude the possibility of a defect in the transport of precursor amino acids into the brain.

Our results for the analysis of vCSF for each patient are contained in Tables 6 & 7. The average concentrations for the vCSF concentration of each of the biogenic amines in this study are compared to literature values of non PD patients in Table 9.

5-Hydroxytryptophan (5-HTP) vCSF Concentrations

The average concentration of 5-HTP in type A patient vCSF was found to be 14.1ng/ml, which falls in the normal range of 1.1 to 68.9 nanomoles per liter, (0.24 to 15.2ng/ml) described by Koskiniemi et al., (1985). In contrast our type B patient VCSF average 5-HTP concentration was found to be some 34 times higher than normal at 525ng/ml. Our standard error was slightly higher than half the mean at 266ng/ml, and the range of values was between 1.2 to 3083ng/ml. This would indicate that we have potential outliers in the data.

Serotonin (5-HT) vCSF Concentrations

We found a significant difference between the concentrations of serotonin in the VCSF of type A and B patients ($p<0.045$). Type B levels were, on average, 50% lower than that of type A patients (0.83ng/ml and 1.65ng/ml respectively). The average concentration of 5-HT in type B patient vCSF was close to the bottom 1/6th of the normal range (0.34-4.8ng/ml) reported by Koskiniemi et al., (1985), whereas the average type A concentration was above the bottom 1/3rd. The standard deviation for the results we

obtained was less than 26% of the mean for type A, and 20% of the mean for type B.

5-Hydroxyindoleacetic Acid (5-HIAA) vCSF Concentrations

There was no significant difference between level of 5-HIAA between type A and B patients ($p<0.445$). The values we obtained for the concentration of 5-HIAA in both type A and type B patients (104.4 and 105.7ng/ml respectively) were at the high end of the range for normal vCSF reported by Koskiniemi et al., (85.2-105.2ng/ml), and in the top third of values reported by Bouckoms et al., (60-120ng/ml) (Bouckoms et al., 1993). Many reports have shown there to be a significant decrease in the amount of HIAA in the lumbar CSF of Parkinson's patients, in particular those with depression symptoms (Kostic et al., 1987, Mayeux et al., 1984). Toghi et al did not find significant reductions in the level of 5-HIAA in their patients, and they postulated that it may be due to presurgical treatment (Toghi et al., 1993).

Homovanillic Acid (HVA) vCSF Concentrations

We found no significant difference between the vCSF HVA concentrations of type A patient and type B patients ($p<0.294$). The average values we obtained, (1007.5ng/ml and 882.6ng/ml type A and B respectively), were outside of the normal range of 150-550ng/ml reported by Bouckoms et al., (1993). We expected somewhat elevated HVA levels (the end product of dopamine catabolism) since our patients were supplemented with L-dopa, the immediate precursor to dopamine.

3-Hydroxykyneurenine (3-OHK) vCSF Concentrations

We found no significant difference between type A and B patient vCSF levels of 3-OHK ($p<0.376$). There is no available data for vCSF 3-OHK levels in the literature. Toghi et al., (1993) reported lumbar CSF levels of 3-OHK decreased significantly in the PD patients as opposed to their controls ($p<0.05$), with concentrations of 0.24 +/- 0.16ng/ml and 1.37 +/- 0. 63ng/ml for PD patients and controls respectively. Our values for vCSF 3-OHK concentrations were 7.11 and 7.81 ng/ml for type A and type B patients respectively indicating that the concentration of 3-OHK in vCSF is much greater than in lumbar CSF. The concentration of 3-OHK did not correlate with levels of 5-

HTP (R squared = 0.127). With high levels of 5-HTP one might expect an increase in the preferential conversion of tryptophan to 3-OHK rather than 5-HTP, since they both use tryptophan as a precursor, and if 5-HTP is accumulating then more tryptophan may become available to the kynurenine pathway and hence lead to the production of more 3-OHK.

Limitations of vCSF Analysis

Like lumbar CSF, vCSF studies have their limitations too. Direct CSF measurements represent amine metabolism at a given time only and not during a definite period such as when the patients symptoms were rated. Some monoamine metabolites are not entirely transported into the blood via the CSF, estimated at about 90% for 5-HIAA (Meek and Neff., 1973), and even less for HVA (Sourkes., 1973). As mentioned earlier, CSF analysis in inherently non-topographical, hence only broad statements of brain monoamine metabolite concentrations can be inferred. A neurotransmitter decrease in one pathway could cause a clinical manifestation, however the change may be very minor when diluted in the vCSF with the output of other pathways.

Carbidopa Dosage

One must wonder if the administration of Sinemet (a mixture of L-dopa and carbidopa) has anything to do with the high 5-HTP levels found in our type B subtype patients. Carbidopa is a peripheral decarboxylase inhibitor and cannot cross the blood brain barrier (BBB) (Butcher and Engel., 1969). Carbidopa blocks the peripheral decarboxylase in the capillary walls that would otherwise convert L-dopa to dopamine (which cannot cross the BBB). In the absence of carbidopa extremely high doses of L-dopa may saturate the peripheral decarboxylase thereby allowing the opportunity for the non-bound remainder to be actively transported across the BBB into the brain. The BBB itself is not a perfect barrier. At the hypothalamus, pineal and circumventricular organs the BBB is necessarily leaky to allow the effect of certain hormones. It is conceivable that following high doses some carbidopa could leak into these areas and block the decarboxylase of nearby monoamine synthesizing neurons. If we assume a finite amount of decarboxylase then carbidopa brain penetration should cause a build up of L-dopa and 5-HTP in the vCSF, by blocking 5-HTP decarboxylase in the brain, thereby limiting the production of 5-HT. The 5-HT stores could then be depleted

throught use without replacement. We would then expect to see a negative correlation between 5-HTP and HVA levels, however we did not find a significant correlation (R squared = 0.005). A positive correlation between carbidopa dosage and 5-HTP concentration was not found. Hence the 5-HTP build up is not a consequence of carbidopa leakage across the BBB and blocking 5-HTP decarboxylase.

The significant difference between the concentrations of 5-HTP in type A and B patients points to a deficiency in the biochemical pathway converting 5-HTP to serotonin in type B as compared to type A (see Figure 2). Alternatively there may be a regulatory feedback problem of serotonin or 5-HIAA in type B as compared to normal in type A. On the other hand there may be a regulatory feedback mechanism for serotonin and/or 5-HIAA in type B patients that is not present in type A. If the problem was regulatory we would expect to see variations in the levels of 5-HT and/or 5-HIAA depending upon which is used for regulation purposes. We do not know of any 5-HIAA receptor being identified, hence it is more likely that a serotonin feedback problem may have occurred. However since both type A and B serotonin levels are in the normal range (as reported by Koskiniemi et al., 1985), it would seem unlikely that there

is a regulatory feedback problem even though the vCSF serotonin levels are significantly different between subtypes A and B. It would seem more likely that the regulatory mechanism is working since levels of 5-HTP are building up without elevating 5-HIAA levels implying a potential attempt to keep serotonin levels in the normal range.

If serotonin is being produced in insufficient quantities and it is the feedback system mediator then it would seem logical that the system would attempt to produce more serotonin, which means more 5-HTP, and ultimately more 5-HIAA. This is of course based on the premise that 5-HTP, 5-HT and 5-HIAA concentrations in vCSF are dependent upon the concentration of their precursors. In a closed system this would be correct since they are all in the serotonin biosynthetic pathway and hence we would expect to see a positive correlation between the concentrations of these metabolites to one another. However, our regression analysis clearly showed no correlation between these metabolites. This implies that there must be mechanisms preferentially removing some of the metabolites from the CSF, and hence ventricular CSF cannot be considered a closed system. This is indeed the case for 5HIAA which is

actively removed from the CSF into the blood stream that permeates the arachnoid villi, and serotonin is taken up by both pre and post synaptic membranes of neurons. If the rates of removal of these metabolites from vCSF were very consistent, and the activity of the serotonergic system was constant, then correlation between metabolite concentrations would still be expected. However, also coupled with this is the difficulty in correlating extremely small numbers (such as the concentration we found for serotonin levels) with very large numbers (such as those for the immediate precursor of serotonin, 5-HTP). A very small difference in the serotonin levels must be consistently able to account for a very large change in 5-HTP. The individual variability, normal range and patient symptom heterogeneity clearly preclude the possibility of clear positive correlation between concentration levels in vCSF for these metabolites in our relatively small sample population (26 patients total).

In effect the fact that we are in the normal range for serotonin (even if it is at the low end of the spectrum) and have an over abundance of 5-HTP would tend to lead us away from the notion of feedback problems and towards potential differences in serotonin biosynthesis in type B

patients as compared to type A patients and normal. It could be that tryptophan hydroxylase is overactive and decarboxylation has now become the rate limiting step in serotonin biosynthesis. This would result in over production in serotonin synthesis. Such overproduction could be hidden from analysis of vCSF by concomitant increase in removal of 5-HT and 5-HIAA from the vCSF by the active methods mentioned above. However, such over production could not be hidden from urine analysis of 5-HIAA concentration. It has been shown that urinary 5-HIAA levels are reduced in PD patients not on any drugs as compared to controls (Markianos et al., 1982) though subtypes A and B were not distinguished, and analysis of urine concentrations was not conducted in this study.

Alternatively it is possible that there are lower levels of decarboxylase enzyme present in type B patients hence the decarboxylation step has become the rate limiting step (rather than tryptophan hydroxylation) and thereby causes a buildup of 5-HTP (Jankovic., 1981). This would imply that the decarboxylase is both intact and saturated with 5-HTP in type B patients. Low levels of intact decarboxylase could be due to reduced transcription of the

gene encoding L-amino acid decarboxylase in serotonin neurons.

Possibly there is a deficient level of the L-aromatic amino acid decarboxylase cofactor pyridoxal 5-phosphate (vitamin B6) which might also cause a build up of 5-HTP. Interestingly in advanced type B patients there is a tendency to develop Pellagra, a disease due to lack of vitamin B3 and B6 (Iacono, personal communication). However not all type B patients have Pellagra or even develop it, hence it may be that vitamin B6 deficiency exacerbates the problem rather than causes it per se.

The possibility that the 5-HTP is shunted to the formation of 5-HIAA without changing brain serotonin concentrations, or that exogenous 5-HTP is converted to serotonin in nonserotonergic systems, such as dopaminergic or noradrenergic, may potentially explain the lack of therapeutic efficacy of 5-HTP. Moir and Eccleston., (1968) gave rats acute loads of 5-HTP and found that increase in the concentration of 5-HIAA occurred before any observable change in 5-HT. They hypothesized a kind of "shunt" in which 5-HTP was rapidly metabolized to 5-HIAA without having much effect on concentrations of 5-HT. It may be possible that such a shunt is operating when 5-HTP

concentrations are high in the Parkinson's Disease type B patient resulting in normal levels of 5-HIAA with low levels of 5-HT and high levels of 5-HTP as we have found in this study.

Involvement of 5-Hydroxytryptophan (5-HTP) in Parkinson's Disease Progression

Toghi et al., (1993) found that there was a progressive decrease in serotonin concentrations and unaltered 5-HTP concentrations in lumbar CSF of PD patients when compared to controls. They suggested an increased involvement of 5-HTP decarboxylase with disease progression (Toghi et al., 1993). We found no correlation between the number of years since onset and the levels of 5-HTP (R squared = 0.038), again this might be due to our small sample size, heterogenous clinical symptomology and individual variability coupled with small sample population.

Another possibility for the involvement of abnormal forms of 5-HTP and serotonin in the pathogenesis of PD might relate to their possible neurotoxic effect. The neurotoxicity of 5-HT and 5-HTP is suggested by the strong possibility that they occur as quinones. This is

significant because the quinone form of 6-hydroxydopamine has been found to react with thiol groups of proteins, and it has been postulated that this reaction is one of the mechanisms of its neurotoxic effect, thus a quinone form of 5-HTP and serotonin might have similar neurotoxic effects (Volicer et al., 1985a). High levels of 5-HTP increase the likelihood of quinone formation. Hence it is possible that a decarboxylase problem could lead to the increase of 5-HTP levels and an increase in quinone form of 5-HTP, and thus aid in the rapid progression of type B symptomology by killing the serotonergic neurons.

How the Data Fit the Current Model (Iacono) for PD

The median raphe nuclei (MRN) sends inhibitory serotonin efferents to the subthalamic nucleus (STN) (Dray., 1981), see Figure 9. It has been established that the MRN of PD patients have reduced decarboxylase levels (Lloyd., 1977). Our high 5-HTP levels strongly suggest a decarboxylase problem in serotonin biosynthesis for type B patients. A decarboxylase problem in the synthesis of serotonin would cause a decrease in the amount of inhibition going from the MRN to the STN allowing it to become overactive (Iacono et al., 1995). The STN has been

shown to be overactive in MPTP induced PD primate models (Aziz et al., 1991). The STN glutaminergic efferents to the ventral globus pallidus interna (VGPi) cause over-excitation of the VGPi. The resulting increased inhibitory outflow from the VGPi to the pedunculopontine nucleus (PPN) results in inhibition of the acetylcholinergic efferent in the descending reticulospinal and vestibulospinal axial motor influences, ultimately increasing the classic type B symptoms of postural instability, gait freezing and akinesia (see Table 1). Of course it must be remembered that analysis of CSF is non-topographical hence we cannot say that the increase in 5-HTP that we have observed occurs in the MRN with any degree of certainty. The dorsal raphe nucleus (DRN) also sends axons to the STN but has not specifically been shown to have a decarboxylase deficiency.

Halliday et al., (1990) showed that the postmortem pathological evidence of a significant loss of serotonergic neurons (>99%) in discrete nuclei in the dorsal raphe (pontine nucleus oralis (PnO)) and median raphe in Parkinsonian patients, correlating the neurotransmitter deficiency with the loss of serotonergic neurons (Halliday et al., 1990). We could also suggest that such 5-HTP buildup as we have observed, may occur in these

serotonergic nuclei. Such a buildup may allow the accumulation of 5-HTP in the quinone form (Volicer et al., 1985b) which is neurotoxic and could eventually kill the neuron and cause a decrease in the amount of serotonergic innervation extending from the DRN to target areas including the STN. The resulting decrease in inhibition of the STN would cause the same cascade effect as described above, resulting in classic B type parkinsonism.

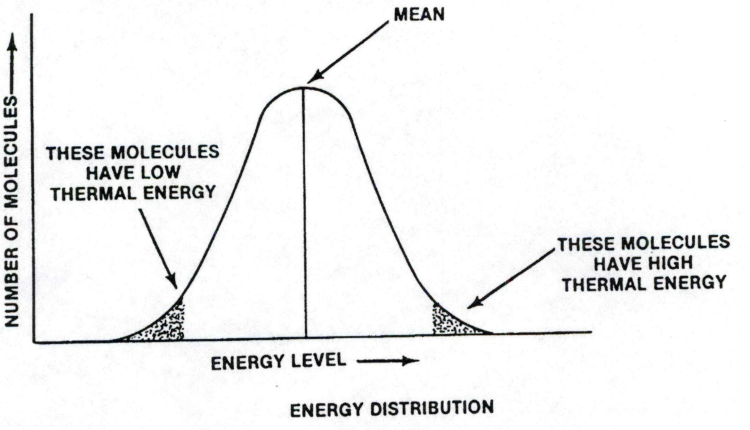
If the decarboxylase deficiency we have proposed is widespread in the raphe, then decreased output of the DRN inhibitory projection to the vGPi would allow excitation of the vGPi, attenuating it. Meanwhile the MRN inhibitory projections to the STN would have decreased output resulting in overactive stimulation of the already attenuated vGPi via the STN. Thus deficient decarboxylase activity throughout the raphe would cause amplification of inhibitory output from the vGPi to the PPN, resulting in classic Iacono type B Parkinson's through descending reticulospinal and vestibulospinal axial motor influences.

APPENDIX

Theory of Operation Of Electrochemical Detection

If the potential of the working electrode is positive with respect to the potential of the electrolyte, the molecule is stripped of one or more electrons (oxidized). If the potential is negative, the molecule is reduced (i.e. the electrode supplies one or more electrons to the molecule). All sample molecules contain the same thermal energy. The energy distribution in a sample solution typically forms a bell curve, where some molecules require more energy than others in order to reach the energy level needed to start the reaction.

Figure 11. Theoretical Graphical Representation of the HPLC-EC Data Output for Range of Retention Times for any Metabolite Under Study.



Note: the area under the curve is calculated and converted to the concentration of the metabolite in the sample solution injected. The area of integration is linearly correlated to the concentrations of standard stock solution run for each metabolite under test.

The following equation expresses the relationship in an electrochemical system:

Total energy = charge * voltage

where the voltage is expressed in electron volts(eV) and the charge is the Faraday constant (96,469 coulombs per mole of electrons).

The current produced is proportional to the amount of analyte injected into the column. This equation describes

the relationship between the current and the amount injected.:

$$i=nKFD2/3C$$

where: i= the current produced by the electrolysis reaction

n= the number of electrons involved in the reaction

F= Faraday constant (96,469 coulombs per mole)

K = cell constant

D = the diffusion coefficient of the analyte

C = the amount of analyte injected into the column

An Electrochemical Reaction
Occurs in Three Stages

1- mass transport (diffusion): the compound diffuses from the solution in the cell to the electrode surface.

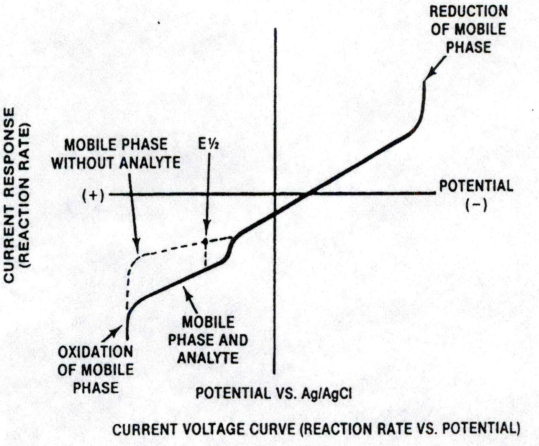
2- Electrolysis: at the electrode surface electrons are either removed from the compound (oxidation) or supplied to it (reduction).

3- Rediffusion: the electrolyzed compound passes back into solution. The slowest of these three steps (known as the limiting factor) controls the rate at which the reaction takes place within the reaction vessel.

Current - Voltage Curves

As the potential applied to the working electrode changes, the current response from the electrolysis of the sample also changes. The response depends on how easily the sample oxidizes or reduces that the different potentials. When the sample oxidizes, a positive current response results; a reduction reaction produces a negative response. This relationship between current and applied potential is called current-voltage curve, or hydrodynamic voltammogram. The current response from the electrolysis depends directly upon the reaction rate. Plotting the current response at different applied potentials shows the rate of reaction versus potential (note the current-voltage curves rarely pass through zero) as shown in Figure 12 below.

Figure 12. Reaction Rate vs Potential Across the Ag/AgCl Electrode HPLC Analysis.



Note: redox reaction of the metabolite under study as it passes through the potential difference of the silver/silver chloride electrode cause a deflection in the current response. The amount of deflection is proportional to the number of molecules of the metabolite that pass the electrode.

The sloping portions of the curve represent potential ranges where the applied potential controls the rate of reaction. Here the oxidation and reduction (electron transfer) steps are slowest. This is due to the fact that not all of the sample molecules have the same thermal energy, but follow the bell curve distribution (shown in Figure 11). The upper curve of the wave represents the molecules with the lowest thermal energy; these require more energy (higher potential) in order to undergo

electrolysis. The lower curve shows the molecules with higher energy; these require less applied energy to react.

The sloping portion of the curve is the integral of the energy distribution curve. At the plateau portion of the curve, diffusion (mass transport) and convection are the slowest reactions. This level represents the potential at which the electrolysis reactions take place most quickly. Here, there is sufficient energy present for the reaction to take place; therefore, this step does not depend on the applied voltage. The plateau is the preferred potential for operation with the best signal-to-noise ration; operating at the voltage just above the curve is recommended. At the ends of the plot, the current rises exponentially due to electrolysis of the mobile phase itself.

Electrode Aging

As a working electrode ages, the current-voltage curve becomes increasingly positive with respect to a newer electrode. The wave shifts to a higher potential, which means that more potential energy must be applied in order to get the same results. As a reference electrode ages, the opposite effect occurs. The diffusion of Cl- ions out

of the diaphragm forces the potential up, which means that the applied potential must be decreased to achieve the same results.

Half Wave Potentials

The half wave potential ($E^{1/2}$) is the potential at which half of the maximum sample response is shown. The half wave value is independent of the concentration, and the height of the wave is proportional to the concentration of the compound of interest. Different compounds may possess different half wave potentials; thus they can be used to separate and identify compounds. If you are analyzing two compounds with different half wave potentials (at least 150-200 mV difference), you can adjust the applied potential to electrolyze one compound (the one requiring the lower applied potential). The other compound then passes through the cell without reacting. Generally, one should choose the lowest possible setting for their analysis in order to keep undesired peaks out of the your chromatogram and maintain optimum selectivity.

Analytical Cell

The Waters 460 analytical cell contains three electrodes: the reference electrode, the working electrode (encased in Borosilicate glass) and the auxiliary electrode (see Figure 12). Together they afford high reliability & sensitivity, low noise and allow operation at the picogram level per single injection. When the power is turned on the working electrode is connected to the electronics. The detector maintains the potential between the working and the reference electrodes; this difference is equal to the value selected using the front panel push buttons. As the sample flows through the cell, the potential at the working electrode electrolyses the components of interest, thus producing a current from the electron transfer. The auxiliary electrode remains at ground.

The current flowing through the working electrode is converted to the a voltage value. The front panel gain setting and filter values attenuate and amplify this signal before it reaches the recorder/integrator output.

Filter Operation

The active filter in the Waters 460 modifies the time constant setting to form a third order Bessel filter.

Activating the additional filter eliminates high frequency noise components from the analog signal. We selected a filter setting according to the frequency of our signal. If the filter setting is to high, peak heights may be reduced and broadened, or seen as baseline noise and ignored. If the setting is too low, baseline noise may be seen as peaks and integrated.

When the FILTER switch is position #1, the response time of the Waters 460 is equal to the value shown on the TIME CONSTANT switch. When the FILTER switch is set to position #2, a higher order filter smoothes out high frequency signals such as electronic interference or air bubbles (which are eventually flushed out of the cell through the flow through diaphragm in the reference electrode). A Faraday cage integrated into the front panel of the detector aids in protecting the analytical cell from electronic noise and changes in ambient temperature.

Solvent Considerations

Generally electrochemical detection is not useful for normal phase chromatography because non-polar solvents are not well suited to many electrochemical reactions. Non-aqueous solvents such as DMSS, THF and acetonitrile require

the addition of salts such as tetabutylammonium hexafluorophosphate of tetrafluoroborate. These salts can extend the potential limits of the analysis beyond those achieved with aqueous solvents. Since minute amounts of dissolved gas or particulate matter can result in large background currents and noisy baselines, we took precautions to avoid them getting into the system by the following.

1- Filter all solvents through a 0.22um membrane filter, to screen out impurities that a 0.45um filter would let pass through.

2- When selecting the mobile phase, we make sure that a very small amount of electrolyte (0.01-.1M) is present. The electrolyte conveys charge through the analytical cell. Ideally, the solvent should have a high dielectric constant to ensure proper ionization of the electrolyte. Both the electrolyte and the solvent must be chemically inert at the surface of the electrode to minimize background current and preserve the electrode surface. Use fresh solvents, salts and modifiers for all mobile phases.ter quality is extremely important; poor water quality is the most common cause of operating problems. We

always used water that has been both distilled and deionized, as well as free from any electrochemically active organic compounds.

3- We used an all glass filter apparatus when filtering solvents, kept the filter apparatus in a clean place (in a plastic bag, to protect the equipment from dust and other particles, which could be flushed into the solvent. When we filtered our eluent, we always discarded the first 200ml to get rid of any particles left in the filtration system.

4- We thoroughly degassed all solvents since dissolved oxygen tends to reduce at potentials of -0.4 and greater (in the negative direction). Failure to do so would tend to raise the background current until some major component of the mobile phase is reduced. Sparging with helium was employed to assist in degassing.

5- To keep the concentration and thereby the sensitivity stable, we used HPLC grade solvents and recirculated the solvent until the sample concentration caused a loss in sensitivity in the tracing output. We also replaced the solvent every few days and used a closed solvent system (not

exposed to air) and then stabilized the system again.

6- We avoided storing solvents at room temperature for extended periods of time by refrigerated them at 4 degrees Celsius until needed, then sparged them with helium while taking care not to expose the solvents to air.

7- We avoided operating the system with solvents that contain more than 80% organic since running 100% organic solvents through the system may dry out the Teflon insert and cause cracking and thereby destroy our column.

We elected to use hydrochloric acid rather than perchloric acid in our mobile phase since we were already using potassium chloride in the reference electrode and perchloric acid would cause precipitation of potassium perchlorate on the surface of the reference electrode.

Recorder and Integrator Polarity

The rear panel polarity switches control the direction of the signals sent from the waters 460 to the recorder or integrator. We set the integrator polarity so that the

output signal remains positive at all times, and left the integrator offset switch OFF.

We checked the Ework twice a day to check the potential between the working and auxiliary electrodes. Changes in the current would have shown us changes in the quality of our buffer solution. Monitoring our output also provided the ability to check off scale peaks.

Analytical Column

Novapak C-18, 4um, 150 x 3.9mm packed with 4um high-density silica based spheres. The bonded phase is also made from novapak silica.

Guard Column

30 x 3.9mm packed with uBondpak C-18/Corasil sentry guard column.10um irregularly shaped silica gel packing material. The guard column protects the analytical column from particulate and chemical contamination in the mobile phase stream. Contaminants that may damage the analytical column are intercepted by the guard column. Adsorption 4ug to 40ug depending on the nature of contaminants. Pressure limit is 40MPa (400 Atm, 6000psi). Temperature limit is 80C Dead volume of holder assembly 4ul (+connecting tubing for universal guard column).

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